THE RESPONSE OF PHOTOSYNTHESIS AND RESPIRATION OF A GRASS AND A NATIVE SHRUB TO VARYING TEMPERATURE AND SOIL WATER CONTENT

A thesis submitted in fulfillment of the requirement for the Degree of Doctor of Philosophy in Plant Biology at the University of Canterbury by

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STATEMENT OF ORIGINALITY

The work presented in this thesis is, to the best of my knowledge and belief, original. The material has not been submitted, either in whole or in part, for a degree at this or any other university.

Tony Joseph
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LIST OF ABBREVIATIONS

A	Photosynthesis
A_{can}	canopy photosynthesis
A_{grass}	gross photosynthesis by the grass
ANOVA	analysis of variance
A_{sat}	Photosynthesis at saturating irradiance and ambient CO_2
A_{shrub}	gross photosynthesis by shrub
ATP	adenosine triphosphate
C_i	intercellular partial pressure of CO_2
C_s	partial pressure of CO_2 at the leaf surface
D	vapour pressure deficit
D_s	air-saturation deficit at the leaf surface
D_{s0}	sensitivity of g_{sc} to D_s
E_o	temperature sensitivity of respiration- related to overall activation energy
ESA	European Space Agency
E_{ti}	transpiration from the dry plant canopy
E_{ui}	evaporation from the understorey and soil
E_{wi}	evaporation from the wet canopy
F_i	drainage from the root-zone
F_n	net carbon exchange for the ecosystem
GPP	gross primary production
g_s	stomatal conductance
g_{sc}	stomatal conductance to CO_2 transfer
g_{sc0}	residual conductance at the light compensation point
H_a	activation energy
H_d	deactivation energy
IPCC	Intergovernmental Panel on Climate Change
J	electron transport rate
J_{max}	maximal light-driven regeneration of RuBP *via* electron flux
K_c	Michaelis-Menten constants for CO_2
K_o	Michaelis-Menten constants for O_2

kPa	kilopascal
L_{can}	canopy light use efficiency
L_{stom}	relative stomatal limitation on photosynthesis
MfE	Ministry for the Environment
Mg	megagram
NADPH	nicotinamide adenine dinucleotide phosphate
N_{area}	leaf nitrogen per unit area
N_{can}	canopy net carbon exchange
NEEMo	net ecosystem exchange model
NIWA	National Institute of Water and Atmospheric research Ltd.
NPP	net primary production
PAR	photosynthetically active radiation
P_{area}	phosphorous per unit area of leaf
P_i	daily rate of rainfall
Q	irradiance
Q_{10}	The temperature coefficient-a measure of the rate of change of a system as a result of increasing the temperature by 10 °C
R	respiration per unit leaf area
R_{10}	rate of respiration at 10 °C
R_{can}	canopy respiration
R_d	respiration in the light
R_g	universal gas constant
R_{grass}	above ground respiration from grass
R_{shrub}	above ground respiration from shrub
R_{soil}	respiration from the soil surface
RuBP	ribulose 1, 5 – bisphosphate
R_o	respiration rate at the base temperature T_o
S	specific leaf area
Sp	Species
S_v	entropy term for V_{cmax}
T_{can}	canopy transpiration
T_l	leaf temperature
UNFCCC	United Nations Framework Convention for Climate Change

V_{cmax}	maximum carboxylation rate of ribulose 1.5-bisphosphate
W_{can}	canopy water use efficiency
W_i	soil water storage on the first day
W_{max}	maximum soil water storage
W_{min}	minimum soil water storage

ABSTRACT

In New Zealand, native shrubs are considered an important potential carbon-sink in disturbed or abandoned land (e.g., pastoral land that is unsustainable for long-term pastoral agriculture). However, the impact of varying environmental drivers on carbon uptake from photosynthesis and carbon loss from respiration of a developing shrubland remains uncertain. In this study, the effects of both temperature and soil water content (θ) on photosynthesis and respiration were examined under controlled growth cabinet and field conditions in a pasture grass and the native shrub, kānuka (*Kunzea ericoides* var. *ericoides*). The purpose of the investigation was to assess the combined impacts of varying temperature and θ on canopy processes and to disentangle the effects of θ on photosynthesis and respiration for the two different plant types.

A controlled growth cabinet study (Chapter 2) showed that θ had a greater effect on the short-term temperature response of photosynthesis than the temperature response of respiration. The optimum value of θ for net photosynthesis was around 30 % for both kānuka and the grass. Statistical analysis showed that the temperature sensitivity of photosynthetic parameters was similar for both plant types, but the sensitivity of respiratory parameters was different. Reduction in θ induced an inhibition of photosynthetic capacity in both plant types. The response of respiratory parameters to θ was not related to substrate limitations, however available evidence suggests that it is likely to be a species dependent plant mechanism in regulating the cost of maintenance due to reduced photosynthate assimilation and decreasing energy supply to support the activity of respiratory enzymes.

Results obtained from a field study (Chapter 3) showed that photosynthesis and respiration in the grass and kānuka were sensitive to seasonal changes in temperature and θ. Photosynthetic parameters showed little acclimation following changes in seasonal growth conditions. In contrast, respiratory parameters tended to acclimate more strongly. Respiratory

acclimation to multiple environmental conditions was characterised by changes in temperature sensitivity and a shift in the response of respiration to temperature, demonstrating the involvement of both 'Type I' and 'Type II' acclimation in both plant types.

The results from controlled growth cabinet and field studies were used to drive a leaf level model that integrates the responses of photosynthesis and respiration to changes in temperature and θ and incorporates acclimation using variable photosynthetic and respiratory parameters (Chapter 4). This model was used to estimate the annual canopy carbon exchange of the grass and kānuka in response to seasonal changes and to predict changes in canopy carbon exchange under varying future climate change scenarios. The model highlighted the importance of considering seasonally-acclimated parameters in estimating canopy carbon exchange of both plant types to concurrent changes in multiple environmental variables.

The overall results support the conclusion that understanding the combined effects of environmental variables on canopy processes is essential for predicting canopy net carbon exchange of a pasture-shrub system in a changing global environment. It has been shown here that the rate of increase in photosynthesis with increasing θ is greater than that of respiration which results in a progressively greater apparent carbon gain at moderate values of θ. Moreover, the impact of lower values of θ, which reduced the apparent sensitivity of respiration to temperature, may effectively decrease the rate of respiration during warmer summer months and enhance thermal acclimation *via* downregulation of respiration. Therefore, considering the influence of soil water conditions on the temperature sensitivity of photosynthetic and respiratory model parameters has important implications for precisely predicting the net carbon exchange of a pasture-shrub system.

CHAPTER 1

Introduction, review of literature, and rational

Chapter 1

1.1 INTRODUCTION

1.1.1 Global carbon budget and climate change impacts

The scientific community has recognised widely that over the last 150 years, the concentration of atmospheric levels of carbon dioxide (CO_2) has risen from 270 to ~389 ppm (NOAA-ESRL 2011) mainly attributable to human activities, including burning fossil fuels, converting forests and grasslands to agriculture and other low biomass ecosystems, soil tillage, land degradation and industrialisation (Vitousek *et al.* 1997; IPCC 2007; Rosenzweig *et al.* 2008). Carbon dioxide is the most prominent greenhouse gas, comprising nearly 76 % of greenhouse gases in the earth's atmosphere.

The future increase in atmospheric carbon dioxide due to increasing human activities is predicted to result in a concomitant increase in average global temperatures resulting from the 'greenhouse effect' (a warming mechanism in which the thermal radiation from a planetary surface is absorbed by greenhouse gases and is re-radiated in all directions) (ESA 2001). Analysis of temperature data collected over the last century shows that the global mean surface temperature has risen by 0.74 °C ± 0.18 °C and the rate of warming over the last 50 years is nearly twice that of the last 100 years (Trenberth *et al.* 2007). According to the Intergovernmental Panel on Climate Change (IPCC 2007), the projected global average surface warming assessed from a hierarchy of models, the global surface temperature is likely to increase further by 1.1 to 6.4 °C at the end of the 21st Century. It has been identified that the increase in average global temperatures over the past several decades is often associated with changes in a number of components of the hydrological cycle and hydrological systems including drought (Bates *et al.* 2008). This strong evidence of climate change highlights the need for action to reduce carbon emission and to identify potential mechanisms capable of adapting to predictable impacts of climate change. While about 40 % of human emissions of carbon dioxide remain in the

atmosphere, half of the remaining is absorbed by the oceans and half by the terrestrial ecosystems (Schimel *et al.* 2001; Bopp *et al.* 2002; Knorr 2009).

Terrestrial ecosystems absorb carbon dioxide from the atmosphere by photosynthesis and release it during respiration at rates that are both non-linear functions of temperature (Baldocchi 2005). When air temperature increases, the concomitant reduction in soil water content due to evapotranspiration is becoming an important issue under climate change and a rising environmental concern particularly in grassland ecosystems (IPCC 2007). Previous studies reported an increase in plant productivity in response to warming and subsequent long-growing seasons (Rustad *et al.* 2001; Nemani *et al.* 2003). A more recent study has contradicted this notion, reporting that global warming-associated drought will adversely affect plant productivity (Zhao and Running 2010) and the continued rise in global temperature increases the frequency of drought - the resulting combined stress due to excessive heat and water shortage could affect the productivity of forest biomes (Tollefson 2010). Frequent droughts may thus affect the response and existence of several ecosystems that are vulnerable to such events and they are, therefore, a major concern for ecologists. As greater shifts in climatic patterns are projected for the coming decades, the carbon storage capacity of ecosystems in response to unpredictable climate pattern may further weaken the terrestrial carbon sink. Even though more severe water deficit and increasing temperatures are issues of major importance, their combined effect on plants has received relatively little attention. Since terrestrial ecosystems are a critical component of the global carbon cycle, improving our knowledge about the fluxes of carbon between the land and the atmosphere is essential in order to predict accurately the ecosystem response to future climate change.

1.1.2 Mitigation of greenhouse gas emissions in New Zealand

Globally, reducing and/or offsetting carbon dioxide emissions has become a major concern for stabilising its concentration in the atmosphere. The Kyoto Protocol is an important effort towards a global emissions reduction regime that will stabilise emissions by setting binding targets for 37 industrialised countries (the Annex I parties to the protocol) to an average of five percent against 1990 levels over the five-year period 2008-2012. Part of the strategy for reducing carbon emission is the active management of terrestrial carbon sink including afforestation and reforestation at a global scale (Schulze *et al.* 2000). The key effectiveness of the Kyoto Protocol relies on understanding the nature of carbon sources and sinks – their distribution, control, longevity and reliability for substantiating the national emission and absorption of greenhouse gases (Group 1998).

New Zealand has committed to develop a national system of inventory for carbon and other greenhouse gases as well as policy to reduce net greenhouse gas emissions through ratifying the Kyoto Protocol and the United Nations Framework Convention for Climate Change (UNFCCC). Importantly, New Zealand has opted to meet its Kyoto Protocol commitments through the offsetting of emissions via sequestration in biomass, following post-1990 afforestation, reforestation and deforestation provisions of Article 3.3 of the Kyoto Protocol for mitigating assigned amounts of emissions during the first commitment period. It has been estimated that forest and shrub vegetation occupy nearly 28 % of the total land area and they are considered the largest above-ground carbon reservoir in New Zealand (White *et al.* 2000). However, forests planted after 1990 on land that was not previously under forest can also earn carbon credits based on their sink capacity. It has been recognised that exotic forests make a significant contribution to maintaining New Zealand's net carbon balance by storing about half of the emissions from using fossil fuels and cement production (MfE 2003; Trotter *et al.* 2004), but the limitations of meeting emission targets using these relatively 'short-duration forests' are well

recognised. Therefore, in order to attain a net reduction in emission of carbon dioxide to 1990 levels, as agreed by New Zealand under the Kyoto Protocol, additional future "permanent" carbon sinks are required.

Shrublands in New Zealand occupy a major part of the total terrestrial plant biomass and the woody shrubland communities currently occupy about 13,200 km^2, which is equivalent to 5.2 % of total land area of New Zealand (Newsome 1987). About half of the total shrublands are principally occupied by New Zealand's most common colonising indigenous shrubland species like mānuka (*Leptospermum scoparium* J.R. et G. Forst) and kānuka (*Kunzea ericoides* var. *ericoides* (A.Rich.) J. Thompson) (Trotter *et al.* 2004). It has recently been recognised that mānuka and kānuka, with high wood density, are a promising carbon sink (Trotter and Fordyce 2006). These shrubland species readily occupy disturbed or abandoned land (e.g., pastoral land that is unsustainable for long-term pastoral agriculture) and accumulate carbon as fast as plantation forests (Scott *et al.* 2000; Tate *et al.* 2000; Tate *et al.* 2003; Trotter *et al.* 2004). Moreover, about 2.6 million ha of unmanaged land are also estimated to be in the process of reversion to indigenous forest (Kerr 2003). Reverting marginal pasture lands to shrublands has several other merits including generating more forest products (e.g. honey) and improving sustainable environmental management, e.g. conservation of biodiversity, erosion control and consequently soil and water conservation (Trotter *et al.* 2005).

As New Zealand agreed to ratify the Kyoto Protocol, the issue of carbon accumulation in shrublands has gained increasing importance, because subsequent development of indigenous forests would result in increasing biodiversity that can act as an active or permanent stand for accumulating carbon over 300-600 year periods (Trotter *et al.* 2005). It has also been argued for inclusion as an additional carbon sink in the national greenhouse gas inventory (Scott *et al.* 2000; Trotter *et al.* 2004). Even though previous studies with shrubs give sufficient information on their ecological (Wardle 1991) and structural characteristics (Egunjobi 1969) and relationship

between photosynthetic parameters and leaf nutrient concentration (Whitehead *et al.* 2004b), data on rates of canopy photosynthesis and respiration in relation to environmental and site-related factors are lacking. Thus, there is an urgent need to identify the environmental factors regulating carbon exchange and storage in this ecologically important shrub, regenerating on a pastoral agricultural land.

1.1.3 Importance of assessment of carbon exchange and storage

Photosynthesis regulates biomass production. Respiration in plants consumes an appreciable amount of carbon fixed through photosynthesis while generating energy for growth. Net carbon gain is the balance between photosynthesis and carbon loss through respiration. However, plants having similar photosynthetic rate may vary significantly in their growth rate and biomass production (Hopkins 2009). Therefore, quantifying the temperature response of photosynthesis and respiration of individual species is essential for comparing its responses to environmental and site related conditions. In addition to above-ground plant respiration, below-ground respiration (comprising the sum of breakdown of soil organic matter due to the activity of microbes and root respiration) releases a considerable proportion (20 – 40 %) of the total terrestrial carbon dioxide emissions to the atmosphere (Raich and Schlesinger 1992).

For grassland reverting to shrubland, we can define the net carbon exchange for the ecosystem, F_n as

$$F_n = A_{grass} + A_{shrub} - R_{grass} - R_{shrub} - R_{soil}$$

Where, A_{grass} and A_{shrub} are gross photosynthesis by the grass and shrub, R_{grass} and R_{shrub} are above ground respiration from grass and shrub and R_{soil} is respiration from the soil surface.

Annually, plants fix about one eighth of atmospheric carbon dioxide molecules through photosynthesis, but nearly the same amount of carbon dioxide is returned through plant and soil

respiration. The balance between these exchanges determines the net carbon sink–source relationship (Pregitzer and Euskirchen 2004; Reich 2010) while changes in major climate change drivers alter the balance between these exchanges and affect the sink-source relationship (Cox *et al.* 2000; Baldocchi 2005; Ciais *et al.* 2005). There is ample empirical evidence that the terrestrial component of the carbon cycle is responding to environmental variations and trends on a global scale (Heimann and Reichstein 2008). According to Schimel *et al.*, (2001), the year-to-year variability in terrestrial metabolism observed in several studies using different measurement methods shows that these interannual variations are likely caused by various activities in the land biosphere rather than the oceans. These mainly result from the effect of climate on carbon exchange and storage processes with short life times (e.g. photosynthesis, respiration, nutrient cycling and forest fire).

Both photosynthesis and respiration are sensitive to temperature and the temperature responses vary between these processes, thereby affecting the balance between them (Berry and Bjorkman 1980; Loveys *et al.* 2003; Atkin *et al.* 2006b; Silim *et al.* 2010). In addition to varying temperatures over short (week-month) and long (annual) time scales, concomitant changes in soil water content may often act as another active environmental driver in regulating carbon sink–source relationship. Physiological adaptation to new environmental conditions (usually termed as 'acclimation') is an effective mechanism in plants to reduce the direct impact of changing environmental conditions on their growth and development and enable them to maintain near constant rates of net carbon dioxide exchange (Atkin *et al.* 2006b). Plant response to temperature and soil water content may vary among biome types with respect to the duration and severity of environmental conditions (Froux *et al.* 2004; Luo *et al.* 2010) and little is known about the mechanisms underlying the acclimation of plants to combinations of different abiotic stresses (Rizhsky *et al.* 2004). Therefore, extensive research is needed in order to understand the effects of environmental factors like temperature and soil water content on plant processes influencing

plant growth and development (e.g. Long and Woodward 1988). Moreover, understanding the changes in carbon exchange between the land and atmosphere is fundamental for developing better climate models (Reich 2010).

To assess the response of plants to environmental changes, three aspects are of importance: (1) short-term response, (2) long-term response and / or acclimation to new conditions and (3) survival. Manipulative experiments regulating soil water content and growth temperature, and experiments under natural conditions, focusing on responses of photosynthesis and respiration to varying seasons, are the two major approaches for investigating the response of terrestrial ecosystem to changing environmental conditions. Manipulative experiments regulating soil water content and temperature tend to provide instantaneous responses while natural experiments studying the response of plants to seasonal changes will provide the long-term response in photosynthesis and respiration. The assessment of carbon cycling with respect to changes in environmental conditions, therefore, requires in-depth knowledge of mechanisms regulating the responses of photosynthesis and respiration to varying climatic conditions.

Recent net primary production (NPP) estimates at the national scale (Trotter *et al.* 2004) indicate that shrublands in New Zealand can contribute an NPP similar to exotic forests because the relationship observed between modelled and validation values of NPP was slightly closer to 1:1. However, initial model-based estimation of the New Zealand carbon balance has identified large carbon losses associated with shrubland ecosystem. Such a potential loss is uncertain and therefore, there is an obvious need to understand and improve the accuracy of net carbon balance calculations within developing shrubland ecosystems. Assessments of the carbon balance within developing shrublands have not been attempted and comparatively little is known about carbon sequestration of native shrub species in New Zealand (Trotter *et al.* 2005). In my research, I therefore attempted to explore the responses to and interactions between potential environmental

drivers of aboveground physiological processes governing carbon uptake and storage in a developing shrubland in the early stages of conversion from pastoral agriculture land-use.

1.2 REVIEW OF LITERATURE

This review deals primarily with the response and acclimation potential of two major aboveground metabolic processes in plants (photosynthesis and respiration) to changes in temperature and soil water conditions. Since these physiological processes are linked closely and they together determine plant carbon balance, it is important to study the combined effect of varying temperature and soil water content on these processes jointly because the response of plants to a combination of two different environmental variables is unique and cannot be assessed from the response of plants to each of these variables separately. In addition, emphasis is given to the changes in plant traits associated with the process of photosynthetic and respiratory acclimation in response to the changes in these environmental variables.

1.2.1 Photosynthesis

Photosynthesis is a key determinant of plant growth, and the rate of photosynthesis changes diurnally and seasonally due to the influence of various environmental factors. The biochemical photosynthetic model of Farquhar et al (1980) is widely used for describing the response of photosynthesis to environment with two major parameters - the maximal carboxylation rate (V_{cmax}) and the maximal light-driven regeneration of RuBP *via* electron flux (J_{max}). These two parameters vary widely among species (Misson *et al.* 2006; Yamori *et al.* 2009; Yamori *et al.* 2010; Zhou *et al.* 2011) and within species with respect to their growth conditions (Berry and Bjorkman 1980; Ferrar *et al.* 1989; Bunce 2000; Fan *et al.* 2011). Previous studies have recognised that carbon uptake varies seasonally not only due to the influence of temperature on these photosynthetic parameters (Leuning 1997; Way and Oren 2010; Egea *et al.*

2011a) but also due to changes in soil water content (Hunt *et al.* 2002; Xu and Baldocchi 2003; Grassi and Magnani 2005; Hu *et al.* 2010), light levels (Niinemets and Tenhuen 1997; Delagrange 2011), atmospheric carbon dioxide levels (Possell and Nicholas Hewitt 2009; Fan *et al.* 2011), varying leaf developmental stages (Wilson *et al.* 2001) and the chemical and structural composition of the leaves (Harley *et al.* 1992; Niinemets and Tenhuen 1997; Niinemets *et al.* 1999). Under field conditions, the interaction of various environmental stress factors on plant photosynthesis is complex (Hamerlynck *et al.* 2000) and therefore the analysis of a particular stress in isolation is very difficult. Although, several studies conducted on the effects of stressful conditions have identified their influence on different photosynthetic parameters (Escalona *et al.* 1999; Flexas *et al.* 1999; Loik *et al.* 2000; Flexas *et al.* 2006), little is known about the extent of a particular stress in impairing plant photosynthesis and how plants cope with such stressful conditions. For predicting the likely response of plant photosynthesis to short-term and long-term variation in temperature and associated changes in soil water content, a complete understanding of the underlying mechanisms regulating these photosynthetic model parameters is essential. Here I focus on the effects of changes in temperature and soil water content on plant photosynthesis.

1.2.1.1 The response of photosynthesis to temperature

The temperature response of photosynthesis varies among species, with C_3 plants generally having optima varying between 15 and 30 °C and arid/desert plants, C_4 and CAM plants having higher optimum temperatures generally ranging between 30 and 40 °C (Larcher 2003). The mechanisms involved in the response of photosynthesis to varying temperatures are complex and involve both non-stomatal (direct) and stomatal (indirect) limitations - a schematic illustration of ‘direct’ and ‘indirect’ effects of temperature on leaf photosynthetic mechanisms (Lloyd and Farquhar 2008) is shown in Figure 1.1.

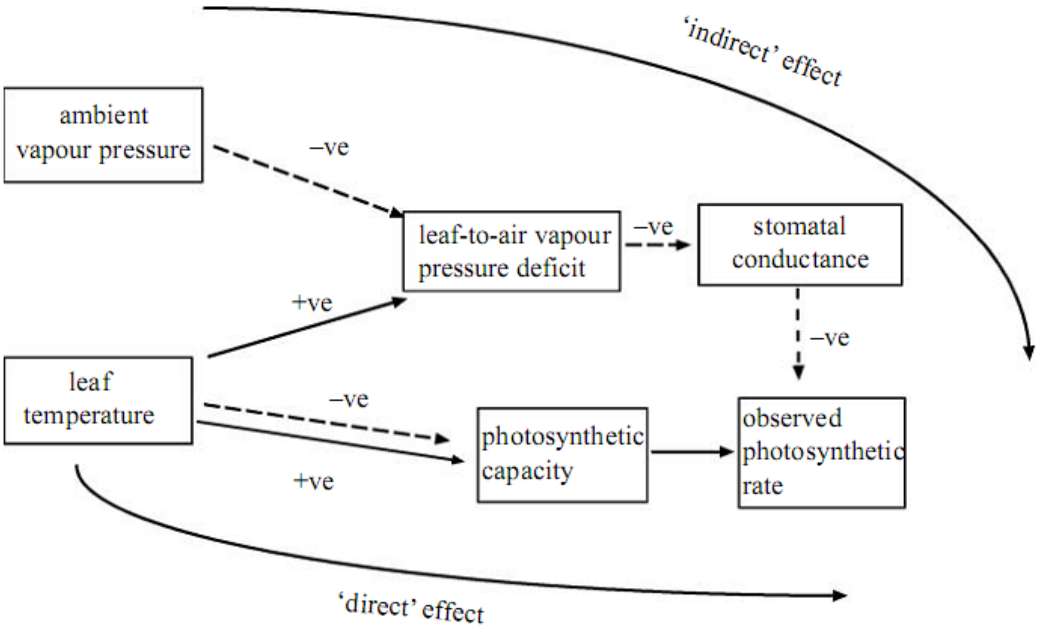

Figure 1.1 Schematic illustration of the effect of temperature on leaf photosynthesis (Lloyd and Farquhar 2008).

The optimum temperature for photosynthesis is influenced mainly by average day-time growth temperatures (Charles-Edwards *et al.* 1971; Berry and Bjorkman 1980). A wide deviation from the optimum temperature usually inactivates the enzymes involved in photosynthesis (Crafts-Brandner and Salvucci 2000) thereby reducing photosynthesis. Farquhar and von Caemmerer (1982) pointed out that variation in optimum temperature for photosynthesis could result from the changes in the relative values of V_{cmax} and J_{max} in response to growing conditions.

At low temperatures, photosynthesis is reduced due to changes in chloroplast structure and development, reduction in chlorophyll content, changes in activity of photosynthetic enzymes, interruptions in photosynthetic electron transport, and stomatal closure (Berry and Bjorkman 1980; Krol *et al.* 2002; Pallardy 2008). At moderately high temperature, inhibition of photosynthesis is common among various species due to reductions in the amount of soluble leaf proteins due to denaturation (Berry and Bjorkman 1980) and enhanced inactivation of Rubisco enzyme (Feller *et al.* 1998; Law and Crafts-Brandner 1999; Crafts-Brandner and Salvucci 2000; Cen and Sage 2005; Kubien and Sage 2008). This inactivation is likely associated with the

interruption in electron transport capacity due to changes in the properties of thylakoid membranes affecting regeneration rate of RuBP (Berry and Bjorkman 1980; Falk *et al.* 1996; Wise *et al.* 2004; Cen and Sage 2005). At high temperatures, plant growth is limited due to a higher optimum temperature for respiration than for photosynthesis, which affects the balance between the two processes. The reversibility of high temperature inhibition is related not only to the temperature experienced but also to the duration of heat stress (Falk *et al.* 1996). Thus the response of photosynthesis to temperature may vary according to the time-scale on which it is measured (Medlyn *et al.* 2002b).

Temperature influences the rate of photosynthesis both in the short and long-term (Yamori *et al.* 2005). The photosynthetic response to short-term temperature changes is species dependent and varies significantly on a seasonal basis that is strongly influenced by the developmental history relating to the changes in physiological age of the leaf tissue (Falk *et al.* 1996; Medlyn *et al.* 2002b). In cold-sensitive plants (e.g., in tomato), a reduction in photosynthesis due to short-term exposure to low temperature is observed (Martin *et al.* 1981) while an increased capacity of photosynthesis is observed in some winter cereals (Huner *et al.* 1993). Even though stomatal conductance declines at low temperature, it plays a minor role in the low temperature-induced inhibition of photosynthesis - this reduction in photosynthesis is more likely mediated by reduced activity of temperature-limited enzymes like Rubisco (Berry and Bjorkman 1980; Sage and Sharkey 1987; Falk *et al.* 1996). In addition, short-term exposure to low temperature can induce an inorganic phosphate limitation of photosynthesis, affecting carbon metabolism and electron transport due to reduced rates of sucrose synthesis and /or source-sink limitations (Falk *et al.* 1996).

Short-term increase in temperature above optimum temperature can also inhibit photosynthesis by deactivating Rubisco enzymes, destabilising thylakoid membranes, and decreasing the amount of soluble leaf proteins (Berry and Bjorkman 1980). At high growth

temperatures above 30 °C, changes in the structure and properties of chloroplast occur (Smillie *et al.* 1978) while above 45 °C, photosystem II is usually damaged (Sharkey 2005), and this is considered as the most heat-sensitive component of the photosynthetic apparatus (Berry and Bjorkman 1980). The direct injury to the photosynthetic apparatus at moderately high temperatures damages the permeability of thylakoid membranes (Schrader *et al.* 2004), leads to loss of photosynthetic electron transport capacity (Carpentier 1999; Sinsawat *et al.* 2004; Kubien and Sage 2008; Mathur *et al.* 2011; Yan *et al.* 2011) and the ability of Rubisco activase to maintain full activation of Rubisco (Harley *et al.* 1992; Crafts-Brandner and Salvucci 2004). Previous studies illustrating the long-term effect of temperature on photosynthesis have showed that photosynthesis is temperature sensitive and is highly responsive to both diurnal and seasonal changes (Monson *et al.* 2002; Monson *et al.* 2005; Zarter *et al.* 2006).

Long-term changes in the growth temperature of plants can cause a shift in the optimum temperature of leaf photosynthesis and can induce acclimation of photosynthesis by changing the photosynthetic capacity or the temperature response of photosynthesis, or both (Bunce 2000; Medlyn *et al.* 2002a; Onoda *et al.* 2005a; Onoda *et al.* 2005b; Yamori *et al.* 2005; Kattge and Knorr 2007; Sage and Kubien 2007; Kositsup *et al.* 2009). This enables plants to perform more efficient photosynthesis at the new growth temperature (Berry and Bjorkman 1980; Sage and Kubien 2007). (Acclimation of photosynthesis is discussed in detail within section 1.2.1.3).

Differences in the ratio J_{max} / V_{cmax} can be explained by nitrogen partitioning between electron transport components and Rubisco (Yamori *et al.* 2011). When the ratio is increased, an increase in optimum temperature for photosynthesis is obtained (Farquhar and von Caemmerer 1982) by reducing the limitation of photosynthesis due to the regeneration of RuBP at a given temperature (Walcroft *et al.* 1997). Reduction in the J_{max} / V_{cmax} ratio with increasing temperature is due to differences in thermal sensitivity (V_{cmax} has a higher temperature optimum than J_{max}). An increased ratio of J_{max} / V_{cmax} leads to a greater ability for thermal homeostasis of

photosynthesis (Yamori *et al.* 2009) and such changes can be accounted for in models by differences in the energies of activation and deactivation for each parameter (Walcroft *et al.* 1997; Medlyn *et al.* 2002a).

At the leaf level, V_{cmax} increases exponentially with temperature and this temperature sensitivity is described in the term H_{av}, which is related to the activation energy of V_{cmax}. Similarly, J_{max} also increases with increasing temperature and its temperature sensitivity is H_{aj}, which is related to the activation energy of J_{max}. Changes in H_{av} are regulated by several other mechanisms including internal CO_2 conductance (Crafts-Brandner and Salvucci 2004; Hikosaka *et al.* 2006) and the activation state of Rubisco (Hikosaka *et al.* 2006; Yamori *et al.* 2006; Warren 2008). At current partial pressures of atmospheric CO_2, H_{av} plays the major role in determining light saturated net photosynthesis, while at high CO_2 and temperature levels H_{aj} plays the major role (Hikosaka *et al.* 2006; Sage *et al.* 2008). At temperatures beyond the optimum, the activation state of Rubisco decreases, resulting in inhibition of photosynthesis (Crafts-Brandner and Law 2000; Crafts-Brandner and Salvucci 2004). J_{max} also decreases at high temperature (Leuning 2002; Medlyn *et al.* 2002a) but the factors determining its temperature dependence are not clear (Hikosaka *et al.* 2006). The activation energies vary greatly among species and climatic conditions and hence they are considered an important factor for evaluating temperature dependence of photosynthetic rate (Wullschleger 1993; Leuning 1997; Onoda *et al.* 2005a; Hikosaka *et al.* 2006; Way and Sage 2008b). However, biochemical mechanisms and the influence of other environmental factors governing the differences in H_{av} and H_{aj} are not well understood.

Another variable that can influence the response of photosynthesis to temperature is stomatal conductance (g_s) because changes in g_s with temperature could affect C_i (intercellular carbon dioxide concentration) and the optimum temperature for photosynthesis. For a given set of V_{cmax} and J_{max} values, the optimum temperature of photosynthesis increases with C_i (Farquhar

and von Caemmerer 1982). Stomatal conductance is reported to have an optimum temperature similar to that for photosynthesis, so that C_i is relatively constant across temperatures and g_s has little effect over the temperature response of photosynthesis (Farquhar and Sharkey 1982). At high temperature, C_i drops substantially due to reductions in stomatal conductance (Berryman *et al.* 1994). At high temperature, measurement of g_s is often confounded with high vapour pressure deficits and when this is avoided, stomatal conductance may increase with temperatures above the optimum temperatures for photosynthesis (Raschke 1970). Large seasonal variation in soil water content, air temperature and vapour pressure deficit resulted in strong seasonal variation in stomatal control of transpiration in blue oak (Xu and Baldocchi 2003). Stomatal closure can improve plant water use efficiency in grassland by limiting transpiration, thereby indirectly influencing productivity (Polley *et al.* 1993). Recent study has shown that the pattern of seasonal change in stomatal conductance parameters differed from that of photosynthetic parameters in *Populus euphratica* Oliv (Zhu *et al.* 2011). Understanding seasonal changes in stomatal conductance is essential for modelling long-term carbon uptake and energy fluxes (Xu and Baldocchi 2003). However, details on seasonal variation in stomatal conductance with respect to changes in phenology and combined stress conditions are limited.

1.2.1.2 The response of photosynthesis to soil water content

Soil water stress is an important site-specific environmental factor limiting plant performance and yield (Knapp *et al.* 2002; Ribas-Carbo *et al.* 2005; Ghannoum 2009; Xu and Zhou 2011) and depends on its intensity and duration (Lawlor and Cornic 2002; Chaves *et al.* 2003; Flexas *et al.* 2004; Grassi and Magnani 2005; Galmés *et al.* 2007). The response of photosynthesis to soil water content is non-linear, i.e., photosynthesis increases at moderate soil water availability and decreases under severe drought and excessive soil water content (Wei *et al.* 2008; Xu and Zhou 2011). Morphologically, water stress reduces leaf growth and leaf area

(Schulze 1986; Santakumari and Berkowitz 1990). Physiologically, water deficit influences electron transport, photosynthetic phosphorylation and chlorophyll content (Schulze 1986; Tezara *et al.* 1999) but not all physiological processes are affected equally. Reduction in water availability also results in a reduction of photosynthetic capacity (Grassi and Magnani 2005; Diaz-Espejo *et al.* 2006; Wei *et al.* 2008; Xu and Zhou 2011). Like drought, excessive soil water content can also result in drastic reduction of photosynthesis (Lavinsky *et al.* 2007; Mielke and Schaffer 2010). Excessive soil water content causes disturbance to hormone signals, oxidative damage and accumulation of toxic products of anaerobic metabolism (Lavinsky *et al.* 2007; Mielke and Schaffer 2010) and reductions in phloem transport (Else *et al.* 2009) causing negative affects on photosynthesis, stomatal conductance and PSII functionality (Xu and Zhou 2011).

Lawlor and Cornic (2002) reviewed the response of photosynthesis of higher plants to soil water deficit and concluded that decreasing relative water content in leaves slows assimilation rate and decreases the potential rate of photosynthesis. Most of the findings showed that photosynthetic reduction due to water deficit is attributed to both stomatal and biochemical limitations, in which the proportional contribution of the latter may increase with severity (Grassi and Magnani 2005; Ripley *et al.* 2007; Ripley *et al.* 2010).

Flexas and Medrano (2002) suggested that in non-stomatal limitation of photosynthesis (Rubisco acivity) was of little importance in the drought-induced limitation of photosynthesis, while decreased capacity for RuBP regeneration due to decreased ATP synthesis would constitute the major role. However, decreased activity of Rubisco is reported with decreasing soil water content in several other studies (Parry *et al.* 2002; Tezara *et al.* 2002; Bota *et al.* 2004; Galmés *et al.* 2011).

Even though several studies have investigated and made progress in understanding plant responses to deceasing soil water content (Chaves *et al.* 2003), the severity of deficit in soil

water content on carbon uptake is not quantified accurately (Jones 2007). The influences of soil water stress on plant photosynthesis mostly depend on the species and genotype, the duration and severity of the stress, the age and stage of plant development and the stress history of the plants (Hsiao 1973; Bray 1997; Hsiao 2000). Plants reduce drought sensitivity by modifying their gene expression (Flexas *et al.* 2006), increasing their levels of membrane permeability and lipid peroxidation in leaves (Luo *et al.* 2010) and adjusting biochemical processes (Yordanov *et al.* 2000; Lambers *et al.* 2008). All these factors enhance water stress tolerance, which leads to acclimation to unfavorable conditions. Studies on the severity of water stress showed that slowly imposed soil water stress can reduce physiological impairment and induce better acclimation than rapidly imposed water stress in different species (Flexas *et al.* 2006; Jones 2007; Kim and van Iersel 2011). Since the temporal pattern of soil water content is influenced by rainfall availability and increased variability in soil water content can significantly affect the carbon cycling processes in grassland (Knapp *et al.* 2002), it is important to understand how seasonal changes in soil water content impose changes in photosynthesis and the mechanisms involved in regulating this process.

1.2.1.3 Photosynthetic acclimation to temperature

Photosynthetic acclimation of plants can occur in response to changing temperature, soil water availability, irradiance or nutrient supply (Turnbull *et al.* 1993; Hikosaka and Terashima 1995; Martindale and Leegood 1997; Bunce 2000; Frak *et al.* 2001; Noguchi *et al.* 2001; Krause *et al.* 2004; Way and Sage 2008b; Dillaway and Kruger 2010; Gea-Izquierdo *et al.* 2010; Delagrange 2011). Photosynthetic acclimation to a long-term change in temperature may result in a change in the shape of the response curve or a shift of the entire curve, thus changing the absolute rate and / or the temperature optimum. This response can be highly variable both within and between species (Atkin *et al.* 2006b). Previous studies have shown that thermal acclimation

is often observed in plants that are exposed to a new temperature in the medium to long term (one week or more), resulting in a shift in the photosynthetic temperature optima (side-to-side) (e.g. *Eucalyptus camaldulensis*, Ferrar *et al.* 1989), translational increase or decrease of rate of photosynthesis (e.g. *Acer saccharum*, Gunderson *et al.* 2000) or both (e.g. *Pinus taeda*, Strain 1976). There is increasing evidence of photosynthetic acclimation to changes in growth temperatures showing that the degree of acclimation may vary from partial or complete when grown under contrasting thermal conditions (Berry and Bjorkman 1980; Bunce 2000; Gunderson *et al.* 2000; Onoda *et al.* 2005a; Weston *et al.* 2007; Dillaway and Kruger 2010; Ghannoum *et al.* 2010; Gunderson *et al.* 2010).

The shift in the optimum temperature of photosynthesis has been found to be associated with changes in the balance between regeneration and carboxylation of RuBP with growth temperature and changes in the balance of these two processes altered the temperature dependence of photosynthesis (Hikosaka *et al.* 1999; Onoda *et al.* 2005a). Increases in photosynthetic rates with thermal acclimation appear to be associated with concomitant increases in proteins regulating photosynthetic capacity (Law and Crafts-Brandner 1999; Campbell *et al.* 2007; Luo *et al.* 2010) and a higher degree of unsaturation of membrane lipids (Murata 1983; Kanervo *et al.* 1997), which consequently alter the balance between RuBP regeneration and RuBP carboxylation reactions (Onoda *et al.* 2005b; Weston *et al.* 2007). Based on changes in the allocation of photosynthetic proteins, Hikosaka (1997) has predicted that plants without plasticity in the ratio of the rate of RuBP regeneration (J_{max}) to the rate of RuBP carboxylation (V_{cmax}) may not acclimate because they exhibit a similar temperature response of RuBP carboxylation limited photosynthesis and RuBP regeneration limited photosynthesis. Onoda *et al.* (2005a) showed that plants with high plasticity of the J_{max}/V_{cmax} ratio may display a greater acclimation potential than those without plasticity of the J_{max}/V_{cmax} ratio. However, further studies on acclimation of photosynthesis to changes in temperature have shown that plasticity in

this balance differs among species or ecotypes (Onoda *et al.* 2005a; Atkin *et al.* 2006b; Ishikawa *et al.* 2007). Recently, Yamori *et al.* (2010) reported that the interspecific differences in photosynthetic temperature acclimation between cold-tolerant and cold-sensitive species are regulated by the plasticity of these photosynthetic parameters.

The thermal acclimation potential of photosynthesis may vary among species and involves different biochemical mechanisms. For example, photosynthetic acclimation to high temperature is reported to result from improved thermal stability of the thylakoid membranes in oak (Haldimann and Feller 2004) and a plastic response of electron transport to environmental temperature in winter wheat (Yamasaki *et al.* 2002). Acclimation to high temperature in spinach, black spruce and creeping bent grass resulted from enhanced thermotolerance of the Rubisco activation state (Yamori *et al.* 2006; Liu and Huang 2008; Way and Sage 2008b). Plants grown in cold temperature had greater amounts of Rubisco and other enzymes than their warm grown counterpart; maintaining greater pools of these enzymes is needed to compensate for their decreased activities at low temperatures (Hurry *et al.* 1995; Åsa *et al.* 1999). Several studies have attempted to clarify the biochemical and molecular mechanisms of temperature responses (Hikosaka 1997; Dreyer *et al.* 2001; Medlyn *et al.* 2002a; Bernacchi *et al.* 2003; Onoda *et al.* 2005a; Yamori *et al.* 2005; Hikosaka *et al.* 2006; Miyazawa and Kikuzawa 2006; Kattge and Knorr 2007; Sage and Kubien 2007; Sage *et al.* 2008; Kattge *et al.* 2009; Kositsup *et al.* 2009; Wang *et al.* 2010; Sandve *et al.* 2011). These studies provided an understanding of the biochemistry of acclimation; changes in gene expression and protein levels associated with acclimation and upgraded mechanisms for improving the modeling of temperature responses.

Photosynthetic variations in the field replicate responses to seasonal variations including soil water content, humidity, and temperature as well as differences associated with phenological developments and canopy position (Ellsworth and Reich 1992; Ellsworth and Reich 1993). The requirement for studies on photosynthetic acclimation to multiple stress factors has long been

recognized (Chapin III *et al.* 1987; Osmond 1987). However, studies evaluating the importance of environmental factors other than air temperature influencing photosynthetic performance and acclimation are limited probably because of the difficulties in analysing and disentangling the combined effects of various factors on photosynthesis. Plants and/or leaves acclimated to water stress have showed maintenance of higher rate of thylakoid electron transport than their non-acclimated counterparts (Kitao *et al.* 2003). A recent study investigating the interaction between high growth temperatures and water stress on gas-exchange properties of *Populus nigra* saplings did not show thermal acclimation in response to high temperatures during water stress (Centritto *et al.* 2011). In another study, an induced high temperature treatment on a desert evergreen shrub, *Larrea tridentata* under well-watered and water-stressed conditions showed reduction in photosynthetic rate in water stressed conditions; however upon release from heat stress, a similar photosynthetic response was observed in both water stressed and well watered plants (Hamerlynck *et al.* 2000). The lack of thermal acclimation of photosynthesis at high temperature under water stress suggests lower carbon uptake than plants growing at high temperature without water stress. Seasonal studies investigating physiological mechanisms of photosynthetic acclimation following a combination of different environmental conditions are limited to answer the uncertainties in the degree and timing of thermal acclimation in plants experiencing seasonal changes in temperature and soil water conditions.

1.2.2 Respiration

Respiration can be divided into two functional components: growth respiration (mainly for yielding energy and carbon skeleton used for generating new plant material) and maintenance respiration for transporting or phloem loading, re-synthesising components of metabolic pathways, regulating ion gradients and altering biosynthetic pathways to adapt to environmental stress (Amthor 1984). Respiration dissipates a major part of the energy captured by the plant

during daily photosynthesis in a biochemically-regulated manner and it may vary among species, ranging from 30-80 % (Ryan 1991; Amthor 2000; Valentini *et al.* 2000; Loveys *et al.* 2002). Since biochemical processes proceed more rapidly at higher temperatures, both photosynthesis and respiration are positively related to temperature. However, respiration is more sensitive to changes in temperature than photosynthesis (Ryan 1991) and therefore, several modelling studies on global warming effects have predicted that respiration will increase at a rate higher than photosynthesis – implying decreased aboveground net primary production (Ryan 1995; Ryan *et al.* 1996b). However, it is uncertain how the complex environmental conditions associated with global warming will affect net carbon balance.

1.2.2.1 The response of respiration to temperature

The rate of respiration increases exponentially in response to short-term increases in temperature owing to the temperature dependence of respiratory enzymes (Atkin and Tjoelker 2003). The shape of this exponential relationship is dependent on both antecedent temperature conditions experienced and the degree of acclimation attained by the plants, which can be rapid, occurring within hours or days (Atkin *et al.* 2000b). The response of plant respiration to temperature is usually represented by changes in two main parameters i.e., basal respiration rate, R_{10} (the rate of respiration at a reference temperature of 10 °C) and the temperature response coefficient (Q_{10} or E_o depending on the formulation of the temperature response function – see section 2.2.4 for equations). Q_{10} is the proportional increase in temperature for a 10 °C increase in temperature. E_o is a term related to the activation energy of the enzymatic reactions in respiration. A constant Q_{10} of 2 is generally assumed in global climate models for all plant species (Ryan 1991; Schimel *et al.* 1997). The sensitivity to temperature actually varies with species and is often influenced by various environmental factors such as changes in measurement temperature (Tjoelker *et al.* 2001; Atkin and Tjoelker 2003), growth temperature

(Atkin *et al.* 2000b; Campbell *et al.* 2007), irradiance (Atkin *et al.* 2000a), seasonal variations (Atkin *et al.* 2000b; Xu and Griffin 2006; Ow *et al.* 2010; Searle and Turnbull 2011), soil water availability (Turnbull *et al.* 2001; Turnbull *et al.* 2002b; Galmés *et al.* 2007; Slot *et al.* 2008; Rodríguez-Calcerrada *et al.* 2010), soil nutrient status (Turnbull *et al.* 2005) as well as plant related factors including species (Turnbull *et al.* 2003; Xu and Griffin 2006; Ow *et al.* 2010), leaf nitrogen content (Turnbull *et al.* 2003), leaf age (Ow *et al.* 2008a; Ow *et al.* 2008b) and canopy position (Griffin *et al.* 2002; Turnbull *et al.* 2003). The Q_{10} of leaf respiration is highly dynamic, because the response of respiration to temperature can vary over longer time as respiratory metabolism acclimates to sustained changes in growth temperature (Atkin and Tjoelker 2003). The values of Q_{10} can vary between 1.1 and 4.2 (Azcón-Bieto and Osmond 1983; Tjoelker *et al.* 2001) and there is growing evidence for differences in the response of plant respiration to differences in the duration and magnitude of temperature change (Atkin *et al.* 2000b; Griffin *et al.* 2002; Xu *et al.* 2007; Ow *et al.* 2008a; Ow *et al.* 2008b; Ow *et al.* 2010). Even though Q_{10} and E_o values are considered as descriptors of the temperature dependence of respiration, they differ with respect to measuring temperature. For example, Zaragoza-Castells (2008) found no seasonal variation in the apparent activation energy (E_o) through the year, despite higher values of Q_{10} in winter than in summer and the constant value of E_o was associated with a Q_{10} declining with increasing temperature. Moreover, Zaragoza-Castells (2008) further demonstrated that moderate temperature dependent variations in the Q_{10} could be accounted for *via* application of single E_o value, with the result that a Arrhenius model successfully accounts for daily and seasonal variations in respirations. Therefore, to accurately incorporate variations in temperature dependence of leaf respiration into large-scale models, a processed-based understanding on changes in E_o or Q_{10} under different environmental conditions is essential. Short-term exposure to temperature change results in an immediate change in the rate of respiration while long-term exposure to a certain temperature may result in acclimation

leading to respiratory homeostasis i.e., the maintenance of identical rate of respiration in plants grown at different temperatures (Figure 1.2). This has been observed in several plant species shifted from a warmer environment to colder environment (Larigauderie and Korner 1995; Atkin *et al.* 2000b; Atkin and Tjoelker 2003; Kurimoto *et al.* 2004; Ow *et al.* 2008a; Tjoelker *et al.* 2008). The long term response usually displays an upward or downward shift in the respiratory temperature response curve with plants are grown at cooler and warmer temperatures, respectively (Lee *et al.* 2005). The degree of acclimation may vary among plant types with respect to their genetic adaptability to the environmental conditions (Larigauderie and Korner 1995; Tjoelker *et al.* 2001) and complete acclimation is often not achievable in many species. However, even partial acclimation may regulate relatively stable carbon levels in plants across a range of temperatures. (The mechanism of acclimation is discussed in detail within session 1.2.2.3).

Since thermal acclimation changes the carbon release through respiration and plays an important role in maintaining carbon balance, ignoring this process may lead to an overestimation of respiration estimated at elevated temperatures and create errors in estimating global as well as regional level of terrestrial carbon exchange in response to climate change (King *et al.* 2006; Atkin *et al.* 2008). Zaragoza-Castells (2008) showed the importance of seasonal acclimation of leaf respiration in determining the variablility of tree growth in both sun- and shade-exposed leaves of *Quercus ilex* experiencing large diurnal and seasonal variation in temperature under dry-land, lower productivity ecosystem. Thus, predictions of the rate of respiration as a function of temperature with changes in other abiotic factors like drought needs knowledge of both temperature and soil water impacts and the rate (and extent) of acclimation.

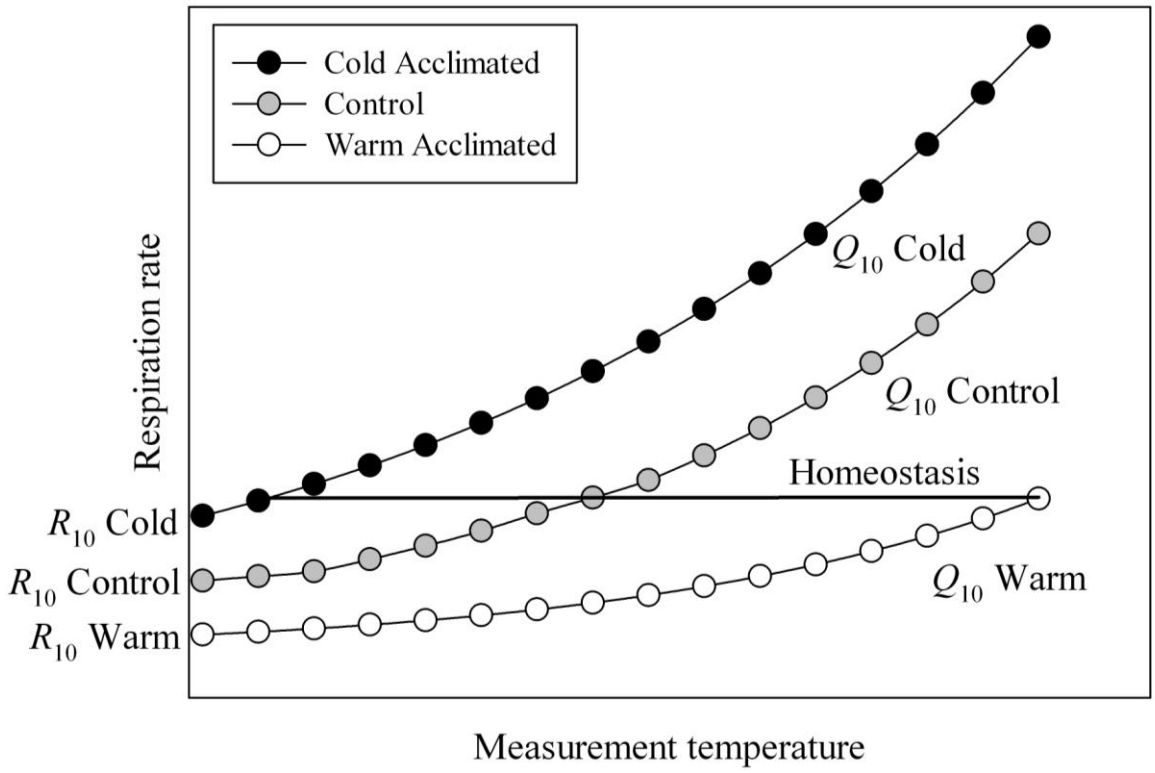

Figure 1.2. Conceptual figure of the response of respiration to temperature demonstrating respiratory acclimation in plants. R_{10} is the rate of respiration at 10 °C which is higher in cold acclimated plants and lower in warm acclimated plants than control plants at a given temperature. Q_{10} is the temperature sensitivity of respiration (the proportional increase in respiration with a 10 °C rise in temperature), which is the slope of the temperature response curve of respiration (shown as dotted lines). Cold acclimated plants have higher slopes and warm acclimated plants have lower slopes than control plants at a given temperature. The solid line indicates the identical rates of respiration in plants at different temperatures, illustrating the concept of thermal homeostasis.

1.2.2.2 The response of respiration to soil water content

Soil water availability plays an important role in regulating plant respiration (Flexas *et al.* 2005). However, the effects of soil water content on plant respiration remain unclear (Gimeno *et al.* 2010). Understanding the sensitivity of respiratory processes to temperature is central for quantifying the climate-carbon cycle feedback (Mahecha *et al.* 2010). The sensitivity of respiration to temperature not only varies with changes in air temperature but also to a considerable extent with changing soil water content (Wen *et al.* 2006). To predict variations in

leaf respiration under field conditions, an understanding is needed on how the rate of leaf respiration at any given reference temperature varies due to the influence of other environmental factors like drought and how this influences the shape of the temperature response of respiration.

Unlike photosynthesis, the rate of respiration may either decrease, increase or change little if measured at a common temperature under water stressed condition, but it will never become totally impaired (Flexas *et al.* 2005). Several studies have shown that water stress can induce a reduction in leaf respiration measured at a common temperature (i.e., R_{10}) (Flexas *et al.* 2006; Atkin and Macherel 2009; Rodríguez-Calcerrada *et al.* 2010). Reduction in the rate of leaf respiration due to water stress is due mainly to reduced substrate supply to mitochondria as a result of reduced photosynthesis, decreased need for respiratory energy for cellular metabolism or growth and decreased abundance, structure and composition of mitochondria (Flexas *et al.* 2006; Atkin and Macherel 2009). However, these responses are mostly species dependent - in sunflower leaves, the rate of respiration increases with severe water stress even though it decreases at early stages of water stress (Ghashghaie *et al.* 2001). The response of respiration in soybean leaves to severe water stress showed a sharp decline in cytochrome pathway respiration rate concomitant with an increase in the alternative pathway respiration rate – there may be little effect on total respiration rate (Ribas-Carbo *et al.* 2005; Flexas *et al.* 2006). The maintenance of respiration in water stressed plants has a negative effect on the carbon balance due to continued or increased release of carbon dioxide into the atmosphere (Atkin and Macherel 2009) but a reduction in respiratory capacity due to water deficit can induce respiratory acclimation (Rodríguez-Calcerrada *et al.* 2010).

The temperature sensitivity of respiration (Q_{10} or E_o) varies in response to seasonal environment changes and is lower in water stressed plants than their fully watered counterparts (Flexas *et al.* 2005). Under field conditions, the sensitivity of leaf respiration to temperature is higher in winter and autumn than in summer in evergreen species e.g., *Chamaecyparis obtusa*,

(Paembonan *et al.* 1991); *Eucalyptus pauciflora* (Atkin *et al.* 2000b); *Pinus radiata* and *Populus deltoides* (Ow *et al.* 2010). The temperature sensitivity of respiration is likely to be highest in tissues with non-limiting availability of respiratory substrates and / or high rates of ATP turnover. Therefore, Q_{10} values have the potential to vary in response to environmentally induced changes in substrate supply (due to drought-induced changes in photosynthesis) and / or energy demand due to water stress induced increase in protein turnover and / or maintenance of ion gradients (Atkin and Macherel 2009).

Turnbull *et al.* (2001) observed a trend towards higher temperature sensitivity of respiration in *Quercus rubra*, *Quercus prinus* and *Acer rubrum* growing at a wetter, lower site than at drier, upper site. In addition, there is evidence that the temperature sensitivity of respiration declines with increasing temperature and decreasing soil water content (Yuste *et al.* 2003; Xu and Baldocchi 2004). Since climate change results in increasing temperature, a reduction in soil water content is also expected due to changes in precipitation and evaporation (Bonan 2008; Heimann and Reichstein 2008) but the impact of varying soil water content on above ground respiration is not clear. In a grassland ecosystem, soil water content is the major environmental factor regulating seasonal variation in ecosystem respiration at constant temperature because of its effect on above-ground biomass (Flanagan and Johnson 2005). However, studies on the combined influence of depleting soil water content and increasing temperature on above ground respiration are limited. There is considerable need for the investigation of effects of increasing temperature and reduced soil water content on above ground respiration and its influence on thermal acclimation.

1.2.2.3 Respiratory acclimation

Previous studies have observed that respiration rate measured in plants grown during the cold season is higher than that of the same plants grown during warm season, when measured at

a common temperature (Reich *et al.* 1996; Atkin *et al.* 2005a; Ow *et al.* 2008a; Ow *et al.* 2008b; Ow *et al.* 2010). Acclimation may result in relatively little seasonal variation in total daily leaf respiratory carbon releases per unit leaf dry mass (Atkin *et al.* 2000b; Ow *et al.* 2010). If the rate of respiration observed in plants at their respective growth temperature is similar when grown at contrasting temperatures, then this phenomenon is termed thermal homeostasis or full acclimation of respiration.

Atkin and Tjoelker (2003) proposed two types of acclimation scenarios in plants and they term them as - Type I and Type II acclimation - to explain the short-term and long-term changes in respiration in response to temperature. In Type I acclimation, changes in growth temperature result in changes in Q_{10} values of respiration with no change in the basal rate of respiration at low temperature - changes in rate of respiration are only at mild to high temperatures. Type I acclimation enables the temperature response of respiration to adjust dynamically to the growing temperature. This type of acclimation occurs commonly in fully developed mature tissues. In contrast, type II acclimation results in changes in respiration at both low and high temperatures, showing an overall shift in the temperature response curve, resulting in a greater degree of homeostasis of respiration than type I acclimation. Thermal acclimation is considered to occur when plants are exposed over longer periods under a new thermal regime (Zaragoza-Castells *et al.* 2007). Type II acclimation is associated largely with temperature-mediated changes in respiratory capacity and basal rate of respiration (Armstrong *et al.* 2006a), while type I acclimation is likely linked to changes in the substrate supply and / or energy demand (Covey-Crump *et al.* 2002). There are studies reporting both type I and type II acclimation occurring in individual plants, following sustained changes in growth temperature (Armstrong *et al.* 2008). Further studies are needed to clarify the extent to which these modes of acclimation occur and their relative contributions under field conditions.

Cold developed leaves are generally thicker than their warm developed counterpart owing to proliferation of cell layers (Atkin *et al.* 2006a; Gorsuch *et al.* 2010). Adaptation to the prevailing ambient temperature is mostly through the adjustment of enzyme activity and substrate availability (Atkin and Tjoelker 2003). Acclimation to low temperature is often associated with an increase in concentration of carbohydrates (Kaplan *et al.* 2004) that is expected to meet the increased demand for respiratory energy associated with cellular maintenance. Sugar content remains relatively high in cold acclimated pre-existing leaves than warm developed leaves (Gorsuch *et al.* 2010).

The speed and degree of respiratory acclimation to temperature may vary both within and among individual species (Tjoelker *et al.* 1999; Loveys *et al.* 2003), under varying environmental conditions (Loveys *et al.* 2003; Armstrong *et al.* 2006a; Atkin *et al.* 2006a; Atkin *et al.* 2006b) and at different developmental stages of the plants (Bruhn *et al.* 2007; Ow *et al.* 2008a; Ow *et al.* 2008b). Among the environmental conditions, the influence of soil water content may have significant impact on respiratory acclimation potential. Bryla *et al.* (2001) showed that the values of Q_{10} decline concurrently with drying soil conditions, displaying acclimation in root respiration to both soil water content and soil temperature. In three deciduous tree species grown at sites with contrasting soil water availability, a higher rate of basal respiration and lower values of Q_{10} were reported at the drier site than wetter site (Turnbull *et al.* 2001). Atkin and Tjoelker (2003) have suggested that low values of Q_{10} are likely linked to reduction in soil water availability because Q_{10} values are lower in tissues where respiratory flux is limited by substrate availability owing to the reduction in photosynthesis. However studies revealing this relationship and adjustments in Q_{10} are limited.

1.2.3 Respiration / photosynthesis ratio

The carbon budget of an ecosystem is the balance between photosynthesis and respiration (Malhi *et al.* 1999). The response of respiration / photosynthesis ratio to growth temperature varies with species (Loveys *et al.* 2002); however, homeostatic respiration / photosynthesis ratio is reported for several plants experiencing contrasting temperatures (i.e. as a result of thermal acclimation of specific rates of photosynthesis and respiration) (Dewar *et al.* 1999; Gifford 2003; Loveys *et al.* 2003). Atkin *et al.* (2007) observed that temperature mediated changes in biomass allocation play a major role in determining whole plant respiration / photosynthesis ratio. Previous studies showed that respiration / photosynthesis ratio is relatively homeostatic at moderate growth temperatures (Dewar *et al.* 1999; Loveys *et al.* 2003; Atkin *et al.* 2006b; Atkin *et al.* 2007). However, homeostasis of respiration / photosynthesis ratio is often disturbed at high or very low temperature (Atkin *et al.* 2005a; Atkin *et al.* 2006b). Ow *et al.* (2008b) showed that thermal acclimation of respiration subsequently reduced the respiration / photosynthesis ratio as temperature increases over a moderate range of temperatures in *Pinus radiata* - this indicates a higher capacity to maintain positive carbon balance when grown at higher temperatures.

Even though photosynthesis and respiration are interdependent, the ratio of photosynthesis and respiration in leaves may vary among species as well as functional groups owing to differences in the temperature sensitivities of these processes (Dewar *et al.* 1999; Gifford 2003; Atkin *et al.* 2006b). The temperature sensitivity of these physiological processes vary under different environmental conditions such as drought (Wen *et al.* 2006; Zhou *et al.* 2007) and changes in plant characteristics including leaf maturity (Armstrong *et al.* 2006a), thereby altering balance between photosynthesis and respiration. There is growing evidence of an increasing respiration / photosynthesis ratio in response to drought (Galmés *et al.* 2007; Slot *et al.* 2008; Atkin and Macherel 2009; Gimeno *et al.* 2010). A high respiration / photosynthesis ratio has recently been reported due to changes in photosynthesis, respiration and isoprene

emission in response to the interaction between high temperature and water stress (Centritto *et al.* 2011). Under water stressed conditions, photosynthesis is impaired and hence carbon balance depends strongly on the response of respiration to water stress (Gimeno *et al.* 2010).

1.2.4 Importance of leaf characteristics on photosynthesis and respiration

Leaf characteristics including leaf age, leaf position, specific leaf area, leaf nitrogen concentration, leaf protein concentration and non-structural carbohydrates concentration are often associated with changes in the rate of photosynthesis and respiration. Rates of photosynthesis and respiration are dependent on the developmental stage of tissues. Respiration rate is higher in developing immature tissues than fully expanded mature tissues (Armstrong *et al.* 2006a; Atkin *et al.* 2007). Similarly, leaf age affects the seasonal pattern of photosynthetic capacity (Bond 2000; Wilson *et al.* 2001; Warren 2006; Han *et al.* 2008; Whitehead *et al.* 2011). As leaves mature, respiration rate decreases with a decrease in the demand for ATP required for growth, changes in protein content and alterations in the density of mitochondria. Nitrogen use efficiency and water use efficiency are relatively higher in mature leaves than either expanding or old leaves (Sobrado 1994). Recent studies have observed that the lower rate of photosynthesis in older leaves is mainly associated with biochemical limitations (Whitehead *et al.* 2011) resulting in reduced concentration of nitrogen and amounts of photosynthetic proteins (Niinemets *et al.* 2005). The developmental stage of leaves has an important role in determining the extent of respiratory acclimation to temperature (Ow *et al.* 2008a).

Canopy position is also an important plant feature regulating various leaf traits in plants. It regulates leaf mass per unit area, nitrogen concentration, protein concentration and non-structural carbohydrates concentration in leaves and reflects on the rate of photosynthesis and respiration (Ellsworth and Reich 1993; Wilson *et al.* 2000; Griffin *et al.* 2001; Meir *et al.* 2002; Turnbull *et al.* 2003; Han 2011). Photosynthesis decreases with increasing depth within the

canopy (González-Rodríguez *et al.* 2001; Wieser 2004) due to canopy light interception and subsequent reductions in irradiance (Meir *et al.* 2002). It is assumed that changes in leaf area alone or in association with leaf nitrogen content can act as a good predictor of photosynthetic capacity (Wilson *et al.* 2000; Meir *et al.* 2002; Walcroft *et al.* 2002). In a recent study, changes in specific leaf area and photosynthetic nitrogen-use effeciency were found to be assiociated with physiological acclimation in hybrid poplar (Benomar *et al.* 2011). In addition, photosynthetic acclimation of the Mediterranean evergreen *Quercus ilex* to progressing drought has been shown to occur through changes in leaf mass per unit area (Limousin *et al.* 2010). Similarly, non-structural carbohydrates (starches and sugars) are often correlated with changes in the rate of respiration associated with leaf temperature (Tjoelker *et al.* 1999; Tjoelker *et al.* 2008; Ow *et al.* 2010). So far, there is no universal interspecific pattern between changes in leaf characteristics and physiological processes that have been observed, and certainly their role in acclimation is not clear.

The concentration of foliar nitrogen is also an important determinant of the rate of photosynthesis and respiration in plants (Lewis *et al.* 2004; Takashima *et al.* 2004). A positive linear relationship between A_{max} and foliage nitrogen concentration on mass as well as area basis is observed in many species (Reich *et al.* 1991; Whitehead and Gower 2001; Grassi *et al.* 2002; Whitehead *et al.* 2004b). V_{cmax} and J_{max} are enhanced with increases in leaf nitrogen concentration while the J_{max} / V_{cmax} ratio decreases with increasing leaf nitrogen concentration (Nakano *et al.* 1997; Grassi *et al.* 2002; Yamori *et al.* 2011). Nitrogen limitations on photosynthesis are determined mainly through nitrogen partitioning between Rubisco and electron transport components (Yamori *et al.* 2011). There is growing evidence that the rate of respiration also increases with increasing foliar nitrogen concentration because of extra requirement for maintenance and repair processes in cells with high nitrogen (Ryan 1995; Ryan *et al.* 1996a; Turnbull *et al.* 2003; Xu and Griffin 2006). Species specific variations in plant

respiration and photosynthesis with foliar nitrogen concentration allow for the scaling of carbon exchange from the leaf to ecosystem level, particularly in forest with mixed vegetation (Whitehead and Gower 2001). Even though several studies (see earlier references) have comparable findings in terms of relationships between leaf characteristics and key physiological processes, not many have extended this relationship to an understanding of the role of changes in leaf characteristics on acclimation of photosynthesis or respiration to varying climatic variables.

1.2.5 Modelling canopy plant carbon fluxes

Models are important tools for understanding forest-ecosystem functions. They are often used to quantify and integrate the interaction of various environmental factors on major plant response processes to evaluate and predict the likely impacts of climate change on forest productivity. This is essential because assessment of the combined effects of climatic factors on forest productivity is not a straightforward process. Models can provide estimates and help with the prediction of future rates of carbon exchange processes at a range of scales from leaf to global levels (Atkin *et al.* 2005b). The level of detail required to parameterise models may vary considerably based on the purpose of the model. Since the interacting environmental factors combine to alter forest productivity the use of simulation models that quantify and integrate the major response processes are regarded valuable only if the assumptions underpinning these models are clearly understood (Medlyn *et al.* 2011).

To understand the impacts of climate changes on forest production and water use, predictions of both carbon and water balances are essential (Morales *et al.* 2005; Gedney *et al.* 2006) and the importance of a coupled-model approach has been reported in several studies (Harley *et al.* 1992; Leuning *et al.* 1995; Kosugi *et al.* 2003). Since coupled-models are based on complex underlying biophysical and biochemical mechanisms, they require evaluation of many parameters including the temperature sensitivity of carbon exchange processes (Leuning

1997; Medlyn *et al.* 2002b; Atkin *et al.* 2008). However, temperature dependence alone cannot explain seasonal change in photosynthetic parameters and hence detailed parameterisation of physiological changes is needed in long-term simulation of gas exchange processes (Kosugi *et al.* 2003). Even though some of these physiological parameters appear similar among particular group of plants (e.g. among C_3 plants, the parameter describing the CO_2/O_2 specificity of Rubisco, which is related to Γ^* (CO_2 compensation point in the absence of dark respiration (μmol mol^{-2})), others are species specific (e.g. V_{cmax}, J_{max} and R_d (mitochondrial respiration in the light (μmol $m^{-2}s^{-1}$) (Farquhar *et al.* 1980)), and therefore the model requires species-specific parameterisation (Walcroft *et al.* 1997). Temporal integration requires predicting diurnal as well as seasonal patterns of physiological processes so that models must accurately describe the dependence of these processes on changes in environmental variables (Walcroft *et al.* 1997).

A recent study using a coupled photosynthesis-stomatal conductance model suggests that to replicate the observed leaf level response of net photosynthesis and transpiration rate to various environmental conditions, seasonal changes in photosynthetic parameters and stomatal coefficients should be considered (Zhu *et al.* 2011). Another recent study showed the importance of considering water stress and the stomatal and non-stomatal limitation of photosynthesis in coupled photosynthesis-stomatal conductance models to precisely represent these functional relationships in relation to depleting soil water content (Egea *et al.* 2011b). However, there is a dearth of information about different mechanisms regulating the seasonal response of gas-exchange parameters when plants experience seasonal changes or acclimate to different environmental conditions.

1.3 RATIONALE FOR THE PRESENT STUDY

From the literature review presented above, it is clear that photosynthetic and respiratory parameters respond to changes in temperature as well as soil water content. The acclimation of

photosynthesis and respiration to temperature can occur in plants over long periods (weeks, months and seasons) but the degree and speed of acclimation to shifting temperatures may vary among species with respect to environmental conditions and the availability of chemical substrates. Photosynthesis and respiration exhibit exponential instantaneous responses to increasing temperature. Beyond the optimum temperature, the rate of both these processes decline. The rate of respiration can increase over a greater range of temperatures than that for photosynthesis because the temperature optimum of respiration is often much higher than that of photosynthesis. When temperature increases, there is a concomitant reduction in soil water content - this is an integral part of seasonality and may cause an opposing effect on photosynthesis and respiration. Relatively few studies have attempted analysing changes in the thermal response of both photosynthesis and respiration simultaneously under varying soil water contents in different plant types. Improved understanding of variability in the temperature sensitivity of photosynthesis and respiration can help explain the influence of changes in other factors (e.g. in this study - soil water availability) affecting both these processes. The major objective of this study was *to resolve the combined effects of short and long-term changes in soil water content and temperature on photosynthesis and respiration in two different plant types.*

It is well documented that a combination of different environmental factors (e.g., heat stress and drought) may have a unique effect on plant growth and productivity compared to each of the environmental factors applied separately (Craufurd *et al.* 1993; Rizhsky *et al.* 2002; Mittler 2006). It is with this in mind that I investigated *the response of photosynthesis and respiration to changes in temperature in a native shrub,* (*kānuka*) *and grass grown under short-term changes in soil water content within controlled growth cabinet conditions* (Chapter 2) *as well as in response to long-term changes in soil water content and temperature under field conditions* (Chapter 3). These studies were conducted to evaluate the role of soil water content in the process of thermal acclimation of both photosynthesis and respiration. My study involved

two plant types - kānuka, a native shrub, and the most predominant C_3 pasture grasses (Browntop and Yorkshire fog) growing in the north Canterbury plain. Both these plant types may exhibit different photosynthetic and respiratory responses to varying temperature and soil water contents. This may likely affect the carbon storage capacity of these plants and the potential of these plant types to act as carbon sinks under future climate change scenarios.

Additionally, the data collected during the course of this research were used with an existing leaf-level model driven by weather data and site variables to simulate the effect of seasonally changing temperature and soil water content on rates of photosynthesis and respiration over an annual period (Chapter 4). Studies modelling the canopy carbon exchange with two different plant types growing in different heights are scarce particularly in a system undergoing land-use change.

1.4 DESCRIPTION OF STUDY SPECIES

1.4.1 Kanuka

Kānuka (*Kunzea ericoides* var. *ericoides* (A.Rich.) J. Thompson) is an erect, woody shrub that grows to 5-7 m or more in height and occurs throughout New Zealand. It is light demanding and more successful on fertile well-drained soil, however it establishes rapidly on disturbed sites and acts as a nurse crop with other early colonising plants thereby assisting the establishment of primary and secondary forests (Wardle 1991). The success of kānuka is attributable to its abundant flowering and production of seeds even when the plants are young, dispersion of seed by wind, the unpalatability of the leaves to grazing mammals and the tolerance of the species to a range of climatic and edaphic conditions (Wardle 1991). Even though the ecology (Wardle 1991) and structural dynamics (Egunjobi 1969) of this species were well documented long ago, the physiological processes regulating carbon uptake in relation to environmental factors in this species is not well understood. Whitehead *et al* (2004b)

investigated the response of photosynthesis of 39-year-old kānuka trees in the field and young trees grown in a nursery to different levels of nitrogen and evaluated the sensitivity of photosynthesis to their leaf nitrogen concentrations to predict its carbon uptake across a wide range of nutritional conditions. A correlation between specific leaf area and leaf nitrogen concentration with annual incident irradiance is also reported for this species (White and Scott 2006). However, understanding of the sensitivity of physiological processes to the combined effects of environmental factors is limited.

1.4.2 Pasture grass

New Zealand pastures are made up mainly of temperate (C_3) grasses (e.g. Ryegrass, Browntop, Yorkshire Fog). Browntop (*Agrostis capillaris* L.) and Yorkshire fog (*Holcus lanatus* L.) are widespread perennial grasses found in both lowland and hill country pastures, and in older pastures in Canterbury. They are tolerant to a wide range of fertility and acidity conditions. In this study, Browntop was selected for the experiment under controlled conditions and Yorkshire fog under field conditions. Clark *et al.* (2001) suggested that the productivity and botanical composition of pastures in New Zealand are likely to be affected by climate change due to changes in temperature, rainfall and CO_2 concentration. Previous studies have analysed the response of photosynthesis in C_3 grasses grown at different temperature levels (Kubien and Sage 2004) and soil water content (Lal *et al.* 1996; Hu *et al.* 2010; Taylor *et al.* 2011). In C_3 grass, a large proportion of the total decline in leaf photosynthesis is due to stomatal limitation under water stressed condition (Taylor *et al.* 2011). White *et al.* (2000) studied the sensitivity of C_3 grass in New Zealand to simulated extreme temperatures and rainfall events and showed that heating treatment had a significant effect on grassland composition, causing a strong decline in live biomass. The sensitivity to heat was high especially when soil conditions are dry (Brock and Kim 1994).

Studies on plant respiration are fewer than for photosynthesis among C_3 grass under different temperature and soil water conditions. A recent study on perennial ryegrass showed that the main source of respiration is through stored reserves (respiratory substrate, specifically the fructan: sucrose ratio) (Lehmeier *et al.* 2010). Searle *et al.* (2010) demonstrated seasonal acclimation of respiration in two alpine grasses growing along an altitudinal gradient in New Zealand with dynamic shifts in the relative abundance of alternative oxidase and cytochrome oxidase proteins. The response of photosynthesis and respiration of C_3 grasses and their acclimation to seasonal changes in temperature and soil water content may have a significant influence on regulating above ground carbon balance. Since the key area of uncertainty in the response of yield and botanical composition of pastures is associated with the intensity of warming (Clark *et al.* 2001), it is important to investigate the sensitivity of above-ground carbon exchange processes to seasonal changes in temperature and soil water content. This will assist in predictions of the future of pastoral agriculture to changing environmental conditions in relation to global warming and climate change.

1.5 OVERVIEW OF THESIS

The thesis is arranged into five chapters covering the following topics.

Chapter 1

General introduction to the background information of this research. The significance of assessment of above ground carbon exchange is discussed along with a literature review on the acclimation of photosynthesis and respiration and their response to temperature and soil water content. This chapter also outlines the aims and overview of this research.

Chapter 2

An investigation was carried out to understand the extent of short-term changes in soil water content conditions on the temperature response of both photosynthesis and respiration in a grass and kānuka. Measurements described in this chapter were carried out on microcosms of grass and two year old kānuka, under different soil water conditions, placed in a controlled growth cabinet condition. The key objective of this chapter was to determine the responses to and interactions between combinations of temperature and soil water content on photosynthetic and respiratory parameters of the two different plant types.

Chapter 3

The influence of diurnal and seasonal variation in ambient temperature and soil water content on carbon exchange processes in the field may differ from their response to induced short-term changes within controlled growth cabinet conditions. Therefore, the key aims of the experiment were to 1) determine the seasonal variation in photosynthetic and respiratory components in the grass and kānuka growing under field conditions; 2) assess the effect of seasonal variation in temperature and soil water content on the potential for photosynthetic and respiratory acclimation over a year; 3) investigate the relationship between long-term changes in photosynthesis and respiration and foliar characteristics.

Chapter 4

A modelling approach was used to incorporate the findings in Chapter 2 and 3 into a coupled photosynthesis-stomatal conductance model. This model integrates the effects of photosynthesis and respiration and incorporates the presence of acclimation to seasonal changes in temperature and soil water content, using variable photosynthetic and respiratory parameters. The model described in this chapter was used to accomplish the following aims: 1) to understand daily and

annual canopy carbon exchange in response to seasonal changes in temperature and soil water content and to find the major parameter regulating canopy carbon exchange; 2) to analyse the sensitivity of the model to changes in photosynthetic and respiratory parameters; 3) to compare estimates of annual net canopy carbon exchange using a static (instantaneous) and a variable (seasonally acclimating) approach; (4) to compare changes in canopy carbon exchange estimates based on field-derived and controlled growth cabinet-derived parameters; (5) to predict changes in net canopy carbon uptake under varying climate change scenarios.

Chapter 5

The final chapter consists of a discussion of the effect of short and long-term changes in temperature and soil water content on canopy carbon exchange processes and discusses areas of possible future research. In this chapter, predicted changes in canopy carbon exchange within a developing shrubland are presented based on the expected climate change scenarios for the next 30 and 80 years in the Canterbury region in New Zealand.

CHAPTER 2

Thermal sensitivity of photosynthesis and respiration changes with varying soil water content in a grass and a regenerating shrub.

Chapter 2

2.1 INTRODUCTION

The physiological processes controlling the exchange of carbon dioxide between the atmosphere and terrestrial ecosystems are influenced by many environmental variables. Net plant carbon gain is determined by rates of photosynthesis and losses from respiration. Variation in environmental conditions can result in immediate changes in the rates of photosynthesis and respiration; consequently, environmental changes have the potential to effect year-to-year variation in carbon exchange and storage of plant organs with short lifetimes (e.g. foliage; Schimel *et al.* 2001).

Soil water content is a major limitation in many plant processes (Knapp *et al.* 2002). It is well known that water held in the soil between field capacity and the permanent wilting point is readily available to plants (Veihmeyer and Hendrickson 1949) but, water availability varies with respect to soil texture and atmospheric demand. Our limited understanding of the impact of soil water content on rates of photosynthesis and respiration limits quantitative assessment of plant growth, primary productivity of ecosystems and the impact of climate change on vegetation. Understanding the response of individual components of an ecosystem to changing environmental variables, and especially soil water availability, is essential for predicting the impact on net carbon exchange within terrestrial ecosystems. This knowledge also informs models incorporating physiological responses in carbon budgets.

Photosynthesis generally increases with short-term increases in temperature and after reaching an optimum temperature it decreases with further increases in temperature (Walcroft *et al.* 1997; Medlyn *et al.* 2002a; Medlyn *et al.* 2002b; Sage and Kubien 2007). The biochemical model proposed by Farquhar *et al.* (1980) analyses the temperature dependence of the photosynthetic rate and assumes that the photosynthetic rate in C_3 plants is limited by the changes in either ribulose 1, 5 – bisphosphate (RuBP) carboxylation or RuBP regeneration. The

optimum temperature of photosynthetic rate is determined by the sensitivity of these two reactions (Hikosaka *et al.* 1999; Yamori *et al.* 2005; Hikosaka *et al.* 2006; Yamori *et al.* 2006; Sage and Kubien 2007; Sage *et al.* 2008). It is well known that the photosynthetic temperature response can change in response to environmental variables and differs among plant species because of differences in the thermal optimum (Hikosaka *et al.* 2006). However, photosynthesis can acclimate to changes in growth temperature, through a shift in the temperature optimum towards the new growth temperature (Berry and Bjorkman 1980; Atkin *et al.* 2006b; Way and Sage 2008b). This occurs not only with the changes in the balance of ribulose 1, 5 – bisphosphate (RuBP) carboxylation or RuBP regeneration (Farquhar and von Caemmerer 1982) but also in response to changes in their temperature dependencies per se (Hikosaka *et al.* 1999; Bunce 2000).

Recent studies investigating the thermal acclimation of photosynthesis have observed varying degrees of acclimation potential in different species ranging from little (Ow *et al.* 2008a; Ow *et al.* 2008b; Dillaway and Kruger 2010) to partial or complete acclimation (Bunce 2000; Gunderson *et al.* 2000). However, the physiological mechanism regulating thermal acclimation in different species is still unclear and the influence of other environmental variables on temperature sensitivity is poorly understood. Since the underlying mechanism involved in this phenomenon is related to seasonal changes in environmental driving variables, a study on the influence of other environmental factors like soil water content on the temperature sensitivity of photosynthetic parameters is essential.

Dark respiration is a complex of metabolic processes and it has been found to be more sensitive to temperature than photosynthesis (Amthor 1991). The thermal response of R is highly dynamic and varies with growth temperature (Criddle *et al.* 1994; Larigauderie and Korner 1995; Atkin *et al.* 2000b; Tjoelker *et al.* 2001; Turnbull *et al.* 2001; Atkin and Tjoelker 2003; Whitehead *et al.* 2004a; Ow *et al.* 2010), species (Larigauderie and Korner 1995), substrate

supply/ adenylate (Atkin *et al.* 2002), light environment (Atkin and Tjoelker 2003), canopy position (Griffin *et al.* 2002; Turnbull *et al.* 2003), nutrient status of the soil (Turnbull *et al.* 2005) and soil water availability (Turnbull *et al.* 2001; Xu and Griffin 2006). Dark respiration often acclimates to changes in long-term growth temperature (Atkin and Tjoelker 2003; Atkin *et al.* 2005a; Atkin *et al.* 2005b; Wythers *et al.* 2005), so that the temperature response of respiration to short-term and long-term changes in temperature is often different. Previous studies have reported that the response of respiration to leaf water content is biphasic i.e., it decreases in the initial stages of water stress and increases with additional stress related demands (Ghashghaie *et al.* 2001; Gulias *et al.* 2002; Flexas *et al.* 2005; Flexas *et al.* 2006). The initial declining trend in respiration rate may be related to a decrease in energy demands for growth and later the increasing trend may be a result of increased metabolism for processes such as osmoregulation or induced senescence. Since different species develop different degrees of water stress under similar conditions, the relationship between leaf respiration and leaf water content differs among species. Experiments conducted with soybean have shown slightly lower respiration under severe water stress was associated with a significant shift of electrons flow from the cytochrome to the alternative pathway (Ribas-Carbo *et al.* 2005). Surprisingly, studies examining the effect of soil water content on physiological characteristics, especially respiration, are very few and a clear understanding of changes in the temperature response of respiration to varying soil water content is lacking. It is important to investigate the degree and extent of respiratory acclimation as influenced by environmental and plant related conditions. This will further enhance the potential for ecosystem modelling.

In this chapter, I report the results of an experiment which demonstrates the effects of short-term changes in soil water content conditions on the temperature response of both photosynthesis and respiration in the grass browntop (*Agrostis capillaris*), a C_3 grass that is common in hill country pastures, and the native shrub kānuka (*Kunzea ericoides*). Kānuka is an

important woody shrub species that occurs naturally with a wide spread distribution in New Zealand (Newsome 1987). Kānuka regenerates on large areas of abandoned hill country pasture (Tate *et al.* 2003). Reversion of grassland to shrubland generally occurs on lands that are marginal for pastoral agriculture and this is considered one of the most important aspects of land-use change in New Zealand. Assessments of the carbon balance within developing shrublands have not been attempted and comparatively little is known about carbon sequestration of native shrub species in New Zealand (Trotter *et al.* 2005). In this research, I therefore attempted to explore responses to and interactions between potential environmental variables for the physiological processes regulating carbon exchange of a developing shrubland in the early stages of reversion. The major objective of this experiment was to investigate changes in temperature sensitivity of carbon exchange mechanisms in response to soil water content within the grass and kānuka.

2.2 MATERIALS AND METHODS

2.2.1 Plant material and growth conditions

Plant material for this experiment was collected from an experimental site at Oxford, New Zealand (latitude 43.3 º S, longitude 172.2 º E, elevation above sea level 34 m). A core sampling technique was adopted for collecting samples of pasture dominated by brown-top (*Agrostis capillaries* L.), Yorkshire fog (*Holcus lanatus* L.) and perennial ryegrass (*Lolium perenne* L.) and brown-top was the grass species selected for the experiment under controlled conditions. Similarly, saplings of two-year-old kānuka (*Kunzea ericoides* var. *ericoides* (A.Rich) J. Thompson) grown within the site were also selected. When the soil water content content was near field capacity, six intact soil cores 300 mm deep and 200 mm in diameter and containing grass and kānuka plants were lifted from the experimental site by inserting PVC pipes into the soil surface and digging around the outer surface of the pipes. The soil at this site is a poorly

drained, Taitapu Typic Orthic Gley (New Zealand Soil Classification) (Hewitt 1998), with a perched water table. Core samples were transported to a glasshouse after covering the basal portion of the core with 2 mm mesh ensuring containment of the soil while allowing excess water to drain. All samples were allowed to establish within a glasshouse for three weeks at ambient daytime temperatures of approximately 25 ºC and night temperature of 15 ºC. Later, all samples were transferred to two growth chambers (Contherm 630, climate simulator, Petone, Wellington, New Zealand) and were allowed to grow within a controlled environment for 10 days. The chambers were set at a constant photosynthetically active irradiance of 720 μmol m^{-2} s^{-1} and photoperiod was maintained at 8/16 hours dark/light using 400W metal halide lamps. Relative humidity and temperature were set at 80 % and 20 ºC during light hours and 70 % and 10 ºC during dark hours, respectively.

2.2.2 Treatments

To create high soil water content, core samples were immersed in water for approximately eight hours. Then they were allowed to drain to field capacity overnight. The core samples were then allowed to dry over a period of 10-12 days within the growth chambers through evaporation from the soil surface and transpiration. Volumetric soil water content (θ) measurements were recorded daily using an automated soil moisture sensor (Model ML2x ThetaProbe, Delta-T, Cambridge, UK). This sensor determines the root-zone volumetric soil water content using the principles of Time-Domain Reflectometry (TDR) by inserting the soil probe (75mm) vertically into the soil core. Physiological measurements were made at intervals as described below.

2.2.3 Gas exchange measurements

Leaf level gas exchange measurements were made on fully expanded pre-existing leaves at four levels of θ (10, 20, 30 & 40 %) and at five air temperatures (8, 13, 18, 20 and 25 ºC) at each level of θ. Two cross-calibrated portable photosynthesis system (Model LI-6400 with CO_2 control modules Li-Cor BioSciences, Lincoln, NE, USA) with the standard 20 x 30 mm chamber were used initially for measuring the response of rate of photosynthesis (A) to varying intercellular CO_2 partial pressure (C_i) around four hours after the commencement of the day-cycle and later dark respiration around three hours after the commencement of night cycle within the growth chamber. Light was provided by blue-red light-emitting diodes mounted on the top of the cuvette at a constant irradiance (Q) of 1500 µmol m^{-2} s^{-1}. To create A/C_i curves at different temperatures, the external CO_2 concentration (C_a) was reduced automatically in 15 steps (from 120 to 0 Pa) using the CO_2 mixer integrated with the photosynthesis system. Temperature and relative humidity within the growth chamber was regulated to maintain a constant vapour pressure deficit (D) between 0.4 and 0.5 kPa. Similarly, dark respiration measurements were made using the two-photosynthesis system with the same plants kept in darkened growth chambers at night under the same soil water content and temperature conditions as those provided for photosynthesis measurements. At each set temperature, the mean of five measurements of respiration was recorded over 25 to 30 seconds intervals, and these data were used to develop a temperature response curve. Temperatures within the growth chamber and leaf chamber were maintained the same and the leaves were allowed to equilibrate to the new temperature conditions for 20 to 25 minutes before commencing respiration measurements.

All leaf samples selected for gas exchange measurements were retained for measurement of surface leaf surface area by analysing high-resolution photographs of these leaves spread on a calibrated surface using Image-Pro Plus 7.0 software and then the leaf samples were dried for 48 hours at 70 ºC. Dried samples were weighed and powdered in a ball-mill for analysis of leaf

nitrogen concentration with a CNS analyzer (Model NA 1500, CarloErba, Milan, Italy). Starch and sugar content were also determined on dried and ground samples following the method explained by Tissue & Wright (1995). Specific leaf area, *S* of all leaf samples was also calculated from leaf area and dry mass and used to express leaf nitrogen concentration on a leaf area basis.

2.2.4 Data analysis

Photosynthetic parameters, V_{cmax} (maximum rate of RuBP carboxylation) and J_{max} (maximum rate of electron transport driving regeneration of RuBP at saturating irradiance) were determined from the A/C_i response curves by fitting the model equations of Farquhar *et al.* (1980). The rate of assimilation limited by carboxylation (A_c) is given by:

$$A_c = V_{cmax}\frac{(C_i - \Gamma*)}{C_i + K_c\left(1 + \left(\frac{O_i}{K_o}\right)\right)} - R_d \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.1)$$

Similarly the assimilation rate limited by electron transport (A_q) is given by

$$A_q = J_{max}\frac{C_i - \Gamma*}{4(C_i + 2\Gamma*)} - R_d \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.2)$$

Where V_{cmax} is the maximum rate of carboxylation by the enzyme Rubisco under conditions of saturating substrate RuBP, J_{max} is the maximum rate of electron transport at saturating irradiance; K_c and K_o are the Michaelis-Menten constants for CO_2 and O_2, respectively. R_d is the rate of daytime respiration resulting from processes other than photorespiration and Γ* is the CO_2 partial pressure at compensation in the absence of photorespiration.

A_{sat} (net photosynthesis at saturating irradiance and ambient CO_2) was also estimated from the A/C_i curves. The reduction in photosynthesis associated with diffusive limitation of the

supply of CO_2 to the carboxylation site through stomata in the grass and kānuka was also calculated from the A/C_i curves by the method of Farquhar and Sharkey (1982).

The temperature sensitivity of leaf photosynthesis (J_{max} and V_{cmax}) was determined using the temperature response function of Leuning (2002).

$$V_{cmax} = \frac{V_{cmax,0} * exp[\left(\frac{H_a}{R\,T_0}\right)\left(1 - \frac{T_0}{T_l}\right)]}{1 + \exp\left((S_v * T_l - H_d)/(R_g * T_l)\right)} \quad \text{..... (2.3)}$$

Where, R_g is the universal gas constant, T_o is the fixed temperature (here 293 K), T_l is leaf temperature, where H_a and H_d are the activation and deactivation energies and S_v is an entropy term for V_{cmax}, $V_{cmax,0}$ is V_{cmax} at the fixed temperature T_o. A similar equation was used to describe the temperature dependence of J_{max}.

Similarly, respiratory parameters were derived from temperature response curves using a modification of an Arrhenius function described by Lloyd & Taylor (1994) and as explained in (Turnbull *et al.* 2005), where respiration rate (R) (μmol m^{-2} s^{-1}) at a given temperature is given by

$$R = R_o . e^{\frac{E_o}{R_g}\left[\frac{1}{T_0} - \frac{1}{T_l}\right]} \quad \text{..... (2.4)}$$

Where R_o is the respiration rate at the base temperature T_o (here 283 K), T_l is leaf temperature (K), R_g is the gas constant (8.314 J mol^{-1} K^{-1}) and E_o is a parameter related to the energy of activation, which describes the magnitude of the temperature response. Standard non-linear curve fitting to photosynthesis and temperature responses was done using the Marquardt-Levenberg algorithm (Sigma Plot, v8.0 SPSS Inc. Chicago, Illinois).

2.2.5 Model of response to temperature and soil water content

A non-linear mixed effect model (Pinheiro and Bates 2000) was used to describe the response of photosynthetic and respiratory parameters to leaf temperature, T_l (K) and soil water content (θ) following the approach of Brown *et al.* (2009). The model included the temperature response functions of Leuning (2002) for photosynthetic parameters and a modified Arrhenius function as described by Turnbull *et al.* (2005) for respiratory parameters, in addition to a linear function for the θ response as follows:

$$V_{cmax} = \frac{V_{cmax,0} * exp[\left(\frac{H_a}{R\,T_0}\right)\left(1 - \frac{T_0}{T_l}\right)]}{1 + \exp\left((S_v * T_l - H_d)/(R_g * T_l)\right)} \left[\frac{\theta n}{\theta c}\right] \quad \text{..... (2.5)}$$

A similar equation was used for describing the response of J_{max} to temperature and soil water content.

$$R = R_o . e^{\frac{E_o}{R_g}\left[\frac{1}{T_0} - \frac{1}{T_l}\right]} \left[\frac{\theta n}{\theta c}\right] \quad \text{..... (2.6)}$$

Where $\theta_n = \theta / \theta_{max}$, is the normalised value of soil water content (θ), where θ_{max} is the maximum soil water content observed and θ_c is the value of θ above which V_{cmax}, J_{max} and R remains constant. Analysis of the non-linear mixed effect models was performed using R software, v2.11.1, R development Core Team (2010).

2.2.6 Statistical analysis

Results presented are the means (± standard error) of six replicates. Two-way analysis of variance (ANOVA) was used to test the direct effect and interactions of temperature and soil water content on species, photosynthetic parameters, respiration parameters, specific leaf area, nitrogen, phosphorous and carbohydrate content of leaves. Differences were considered significant if probabilities (P) were less than 0.05, using Minitab 15 (Minitab Inc. Pennsylvania,

USA). Least significant difference (LSD) is the post hoc test - run after ANOVA for both the plant types.

2.3 RESULTS

2.3.1 Leaf characteristics

Leaf characteristics, including specific leaf area (*S*), leaf nitrogen concentration, leaf phosphorous concentration and leaf starch and sugar content differed between the grass and kānuka (Table 1). *S* was higher in the grass (10.8 - 33.5 m^2 kg^{-1}) than in kānuka (3.2 - 12.5 m^2 kg^{-1}). The concentration of leaf nitrogen was higher in kānuka (0.98 - 2.70 g m^{-2}) than in the grass (0.63 - 1.70 g m^{-2}). Leaf phosphorous concentration was higher in kānuka (0.06 - 0.40 g m^{-2}) than in the grass (0.04 - 0.10 g m^{-2}). Starch content ranged from 0.32 to 0.60 g m^{-2} in the grass and 0.87 to 5.25 g m^{-2} in kānuka, while sugars ranged from 0.21 to 0.48 g m^{-2} in the grass and 0.37 to 1.3 g m^{-2} in kānuka, respectively. In both the plant types, these leaf characteristics did not display significant variation during the course of a drying cycle.

2.3.2 Photosynthesis

V_{cmax}, J_{max} and the J_{max}/V_{cmax} ratio differed significantly between the grass and kānuka. V_{cmax} ranged between 10.7 and 63.5 µmol m^{-2} s^{-1} in the grass and between 6.04 and 55.2 µmol m^{-2} s^{-1} in kānuka ($F = 14.3$, $P < 0.001$). J_{max} ranged between 35.6 and 130.8 µmol m^{-2} s^{-1} and between 18.5 and 106.7 µmol m^{-2} s^{-1} in the grass and in kānuka, respectively ($F = 38.6$, $P < 0.001$). Similarly, the J_{max}/V_{cmax} ratio ranged between 1.9 and 3.7 in the grass and between 1.7 and 3.6 in kānuka ($F = 11.1$, $P < 0.001$). These photosynthetic parameters were strongly influenced by temperature for both the grass and kānuka and increased exponentially with increasing leaf temperature (Figure 2.1). Changes in leaf temperature from 8 to 25 °C also resulted in a significant change in the balance between RuBP regeneration and carboxylation rate and there was a strong and negative relationship between the leaf temperature and J_{max}/V_{cmax} ratio.

The response of photosynthetic parameters to temperature was significantly influenced by soil water content for both the grass and kānuka and is presented in Figure 2.2 The minimum and maximum values of net photosynthesis at saturating irradiance and ambient CO_2 measured at 20 °C ($A_{sat,20}$) ranged between 5.2 and 14.5 µmol m^{-2} s^{-1} in the grass and between 1.0 and 9.3 µmol m^{-2} s^{-1} in kānuka, respectively. The difference of $A_{sat,20}$ at varying soil water content was significant between the species (P<0.001). The relationship between $A_{sat,20}$ and soil water content was curvilinear (P < 0.001) and it could be described by quadratic regression equations for both the species.

Table 2.1 Leaf characteristics for the grass and kānuka undergoing a drying cycle within a controlled condition. Values shown are means (± standard error) where n = 6. Significance of treatments effect for species (Sp), soil water content (θ, %) and the interaction between species and soil water content (Sp x θ) are indicated as the P-value or as nonsignificant (ns). Different letters within rows indicate statistically different values at P < 0.05 based on least significant difference test of treatment means.

Leaf Traits	**Grass**				**Kānuka**				**ANOVA**	
	Soil Water Content (θ)				**Soil Water Content (θ)**					
	10	**20**	**30**	**40**	**10**	**20**	**30**	**40**		
S (m^2 kg^{-1})	21.5 (1.41) a	23.3 (1.48) a	23.2 (2.00) a	20.56 (0.91) a	6.7 (0.52) b	7.6 (0.64) b	8.2 (0.56) b	8.1 (0.35) b	Sp	P < 0.001
									θ	ns
									Sp x θ	ns
N_{area} (g m^{-2})	1.06 (0.12) c	1.04 (0.09) c	1.15 (0.14) bc	1.17 (0.10) bc	1.67 (0.31) a	1.35 (0.14) b	1.28 (0.12) b	1.29 (0.17) b	Sp	P = 0.008
									θ	ns
									Sp x θ	ns
P_{area} (g m^{-2})	0.08 (0.01) c	0.06 (0.01) c	0.07 (0.01) c	0.07 (0.01) c	0.21 (0.04) a	0.17 (0.04) b	0.17 (0.04) b	0.15 (0.02) b	Sp	P < 0.001
									θ	ns
									Sp x θ	ns
Starch (g m^{-2})	0.38 (0.02) c	0.42 (0.04) c	0.46 (0.05) c	0.48 (0.03) c	1.76 (0.39) b	1.84 (0.39) ab	2.22 (0.69) a	1.85 (0.35) ab	Sp	P < 0.001
									θ	ns
									Sp x θ	ns
Sugar (g m^{-2})	0.34 (0.03) c	0.32 (0.03) c	0.35 (0.05) c	0.36 (0.03) c	1.01 (0.12) a	0.99 (0.06) a	0.84 (0.09) b	0.75 (0.12) b	Sp	P < 0.001
									θ	ns
									Sp x θ	ns

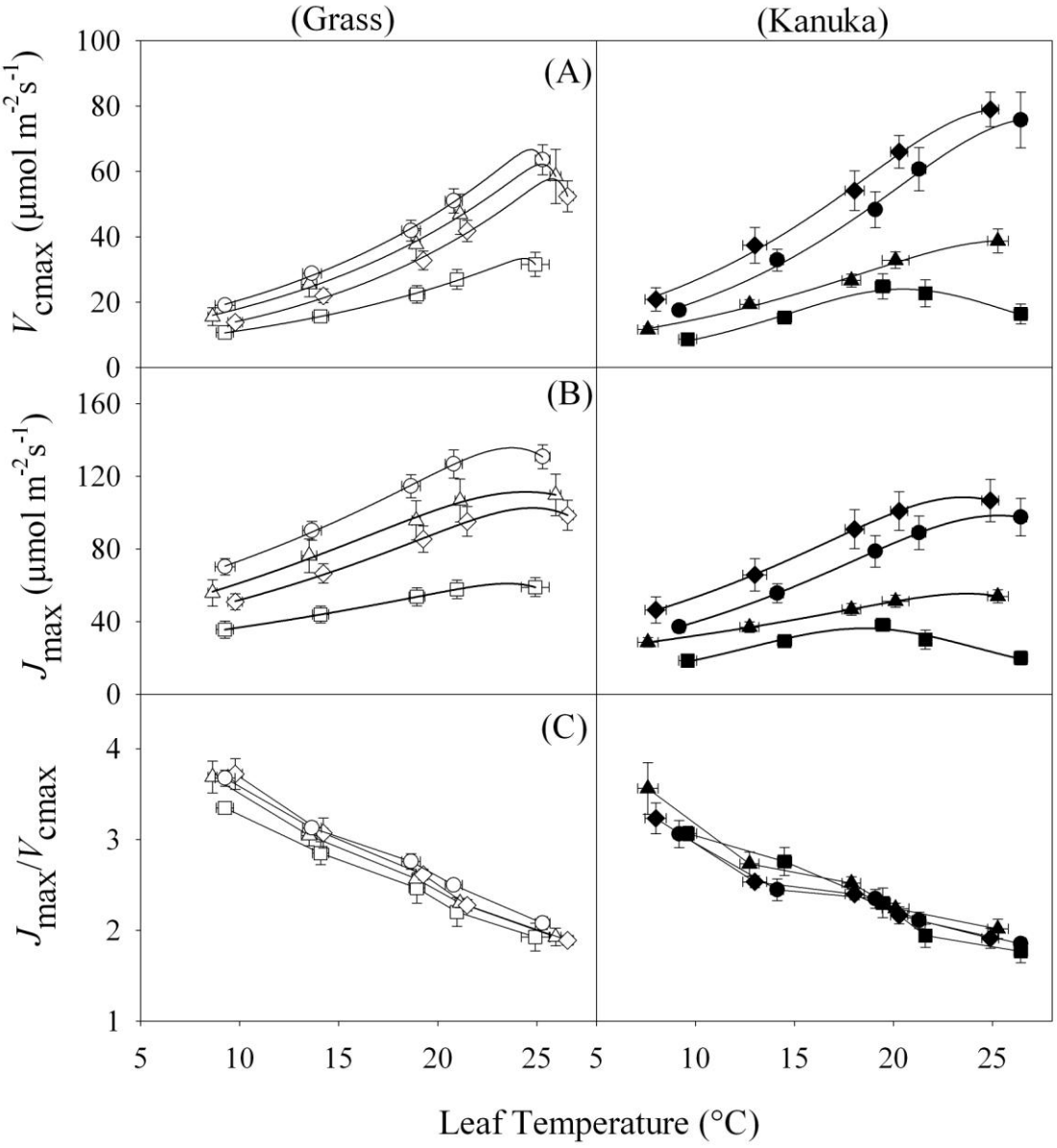

Figure 2.1 (A, B) The relationship between leaf temperature and the photosynthetic model parameters V_{cmax} and J_{max} derived by fitting the model equations of Farquhar *et al* (1980) to A/C_i response curves measured at varying soil water content in the grass (open symbols) and kānuka (closed symbols). The symbols indicate variations in soil water content i.e., 10 % (□), 20 % (Δ), 30 % (◊) and 40 % (○). Curves were fit using the temperature response function of Leuning (2002). Values shown are means (± standard error), where n = 6. (C) The ratio of J_{max}/V_{cmax} plotted as a function of leaf temperature at varying soil water conditions.

Maximum carboxylation rate of Rubisco and the ribulose bisphosphate (RuBP) regeneration capacity mediated by maximum electron transport at 20 °C (i.e., $V_{cmax,20}$ and $J_{max,20}$)

also varied within the species. Low $V_{cmax,20}$ and $J_{max,20}$ values were observed at 10 % soil water content and maximum values were observed near 40 % soil water content for the grass and 30 % soil water content for kānuka. In both the grass and kānuka, $V_{cmax,20}$ and $J_{max,20}$ values showed an approximately linear increase with increasing soil water content. Analysis of variance showed that variation in soil water content from 10 to 40 % resulted in a 196 % and 253 % increase in $V_{cmax,20}$ ($P = 0.001$) and $J_{max,20}$ ($P<0.001$) respectively in the grass and a 220 % and 277 % increase in $V_{cmax,20}$ ($P = 0.001$) and $J_{max,20}$ ($P = 0.001$) respectively in kānuka. Values of the $J_{max,20}$ / $V_{cmax,20}$ ratio fell within a narrow range of 2.28 to 2.61 for the grass and varied significantly at varying soil water contents. For Kanuka, values of $J_{max,20}$ / $V_{cmax,20}$ ratio varied between 2.03 and 2.21 and did not vary significantly at varying soil water contents. The energy of activation (temperature sensitivity) of both V_{cmax} (H_{av}) and J_{max} (H_{aj}) increased with increasing soil water content and declined near 40 % soil water content (Figure 2.3) in both the grass and kānuka. Since the temperature response of photosynthesis was significantly influenced by varying soil water content in both plant species, the combined effects of temperature and soil water content is resolved using a linear mixed effect model. Results of the mixed effect model showed that the values of V_{cmax} and J_{max} of the grass started declining when θ values fell below nearly 17 to 18 % soil water content and when V_{cmax} and J_{max} values declined below about 23.6 % soil water content in kānuka (Table 2.3). Similarly, linear mixed effect model was also used to resolve the combined effect of temperature and soil water content on J_{max}/V_{cmax} ratio.

The relative limitation to photosynthesis imposed by stomatal processes was found to be significantly higher in kānuka than the grass ($P<0.001$; Figure 2.4). Within a range of 8 to 25 °C, the stomatal limitation to photosynthesis varied between 8.5 ± 1.2 % and 30.8 ± 1.6 % in the grass and between 8.2 ± 1.0 % and 49.28 ± 2.6 % in kānuka. Analysis of variance showed a significant effect of temperature in influencing stomatal limitation in both the grass ($P<0.001$) and kānuka ($P<0.001$) and a significant soil water content effect in the grass, but the combined

effect of soil water content and the temperature in both species was resolved using the linear mixed effect model.

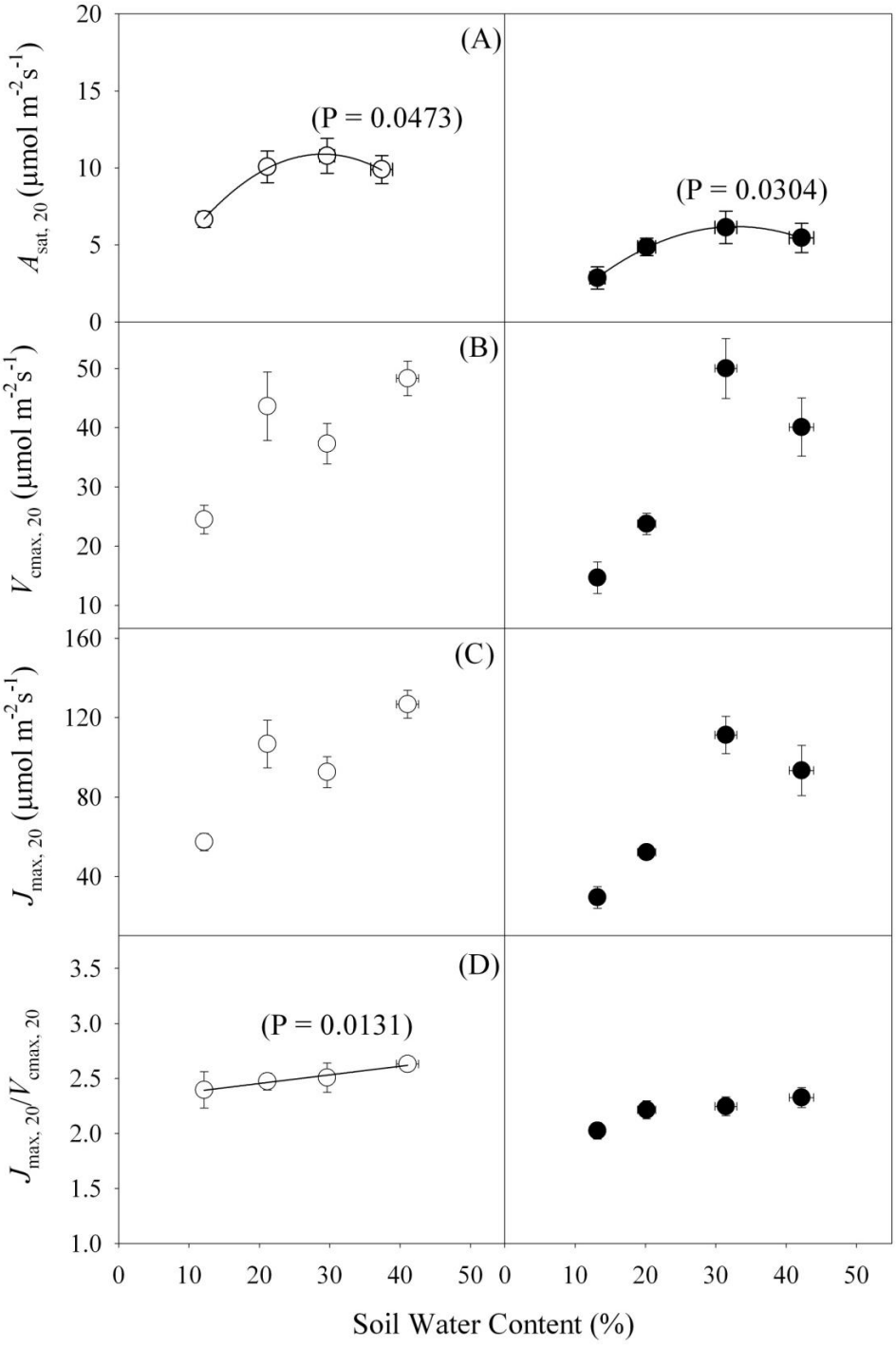

Figure 2.2. Relationships between a range of photosynthetic parameters (at a common temperature of 20 °C) and soil water content in the grass (open circles) and kānuka (closed circles). The significant regression parameters fitting the relationships are presented in Table 2.2. Photosynthetic parameters (i.e., $V_{cmax,20}$, and $J_{max,20}$) were obtained by using the temperature response function of Leuning (2002) on the data in Figure 2.1.

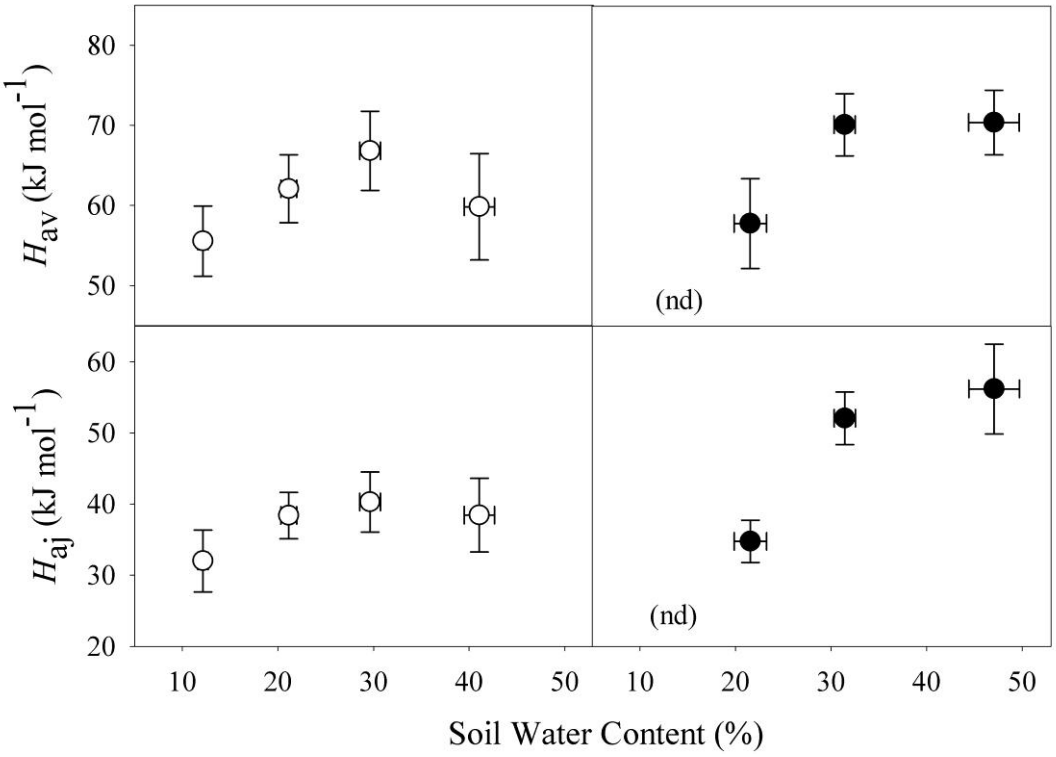

Figure 2.3 Values of parameters describing the temperature response of photosynthesis with energies of activation for V_{cmax} (H_{av}) and J_{max} (H_{aj}) under varying soil water content in the grass (open circles) and kānuka (closed circles). Given the limited temperature response at 10 % soil water content, H_{av} and H_{aj} could not be resolved for kānuka (nd).

Table 2.2 Summary of regression statistics for significant relationships ($P < 0.05$) between photosynthetic parameters and soil water content for the grass and kānuka.

Figure	Regression relationship	r^2
	Grass	
2.2 A	$A_{sat,20}$ (µmol m^{-2} s^{-1}) = -0.0148 θ^2 + 0.8578 θ (%) - 1.5554	0.99
2.2 D	$J_{max,20}$ /$V_{cmax,20}$ = 2.2829 + 0.0871 θ (%)	0.94

Figure	Regression relationship	r^2
	Kānuka	
2.2 A	$A_{sat,20}$ (µmol m^{-2} s^{-1}) = -0.0086 θ^2 + 0.562 θ (%) - 3.0457	0.99

Table 2.3 Parameters obtained by fitting the data to the mixed effect models for V_{cmax} and J_{max} using leaf temperature and soil water content as variables for the grass and kānuka.

Species	Variables	$V_{cmax,\ 20}$ (μmol m^{-2} s^{-1})	H_{av} (kJ mol^{-1})	θ_c (%)	P value
Grass	Leaf temperature, Soil water content	39.72	53.9	17.0	< 0.001
Kānuka	Leaf temperature, Soil water content	35.31	49.4	23.6	< 0.001
Species	**Variables**	$J_{max,\ 20}$ (μmol m^{-2} s^{-1})	H_{aj} (kJ mol^{-1})	θ_c (%)	**P value**
Grass	Leaf temperature, Soil water content	97.72	28.8	18.0	< 0.001
Kānuka	Leaf temperature, Soil water content	78.67	28.1	23.6	< 0.001

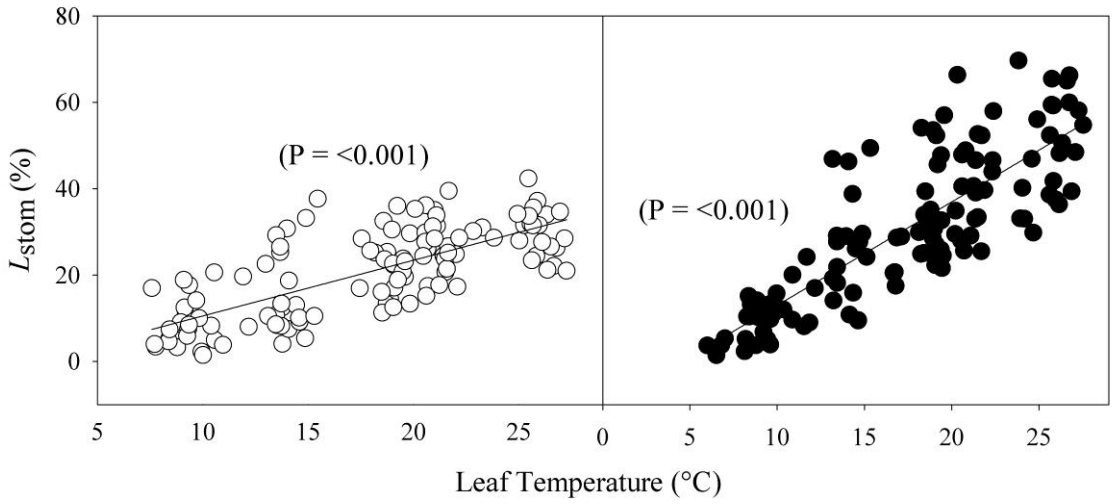

Figure 2.4 The relationship between relative stomatal limitation, L_{stom} (%) and leaf temperature (°C) in the grass (open circles) and kānuka (closed circles) experiencing a drying cycle is described by a line of best fit using linear regression. To resolve the combined effects of temperature and soil water content a linear mixed effect model was used which generated the following relationships: - grass L_{stom} (%) = ((1.0287 x -22.3535) x T_l (°C) +11.5131), r^2 = 0.70 and kānuka L_{stom} (%) = ((2.257 x -12.4626) x T_l (°C) -3.0778), r^2 = 0.70.

2.3.3 Respiration

Respiration increased with increasing temperature in both the grass and kānuka (Figure 2.5). R_{10} was higher for kānuka (range from 0.31 to 0.99 μmol m^{-2} s^{-1}) than for the grass (range from 0.15 to 0.43 μmol m^{-2} s^{-1}). Analysis of variance showed a significant influence of soil water content on R_{10} values in kānuka (F = 5.20, P = 0.008) but not in the grass (Figure 2.6). A linear decline in the energy of activation (E_o) values with increasing soil water content was evident in both species (Figure 2.6). Results of non-linear mixed effect model showed that R_{10} values of kānuka started declining when θ values fell below nearly 15 %. Analysis of variance further showed a significant reduction of sugar content with increasing soil water content within kānuka (F = 4.45, P = 0.018) but the starch content did not vary significantly in either the grass or kānuka.

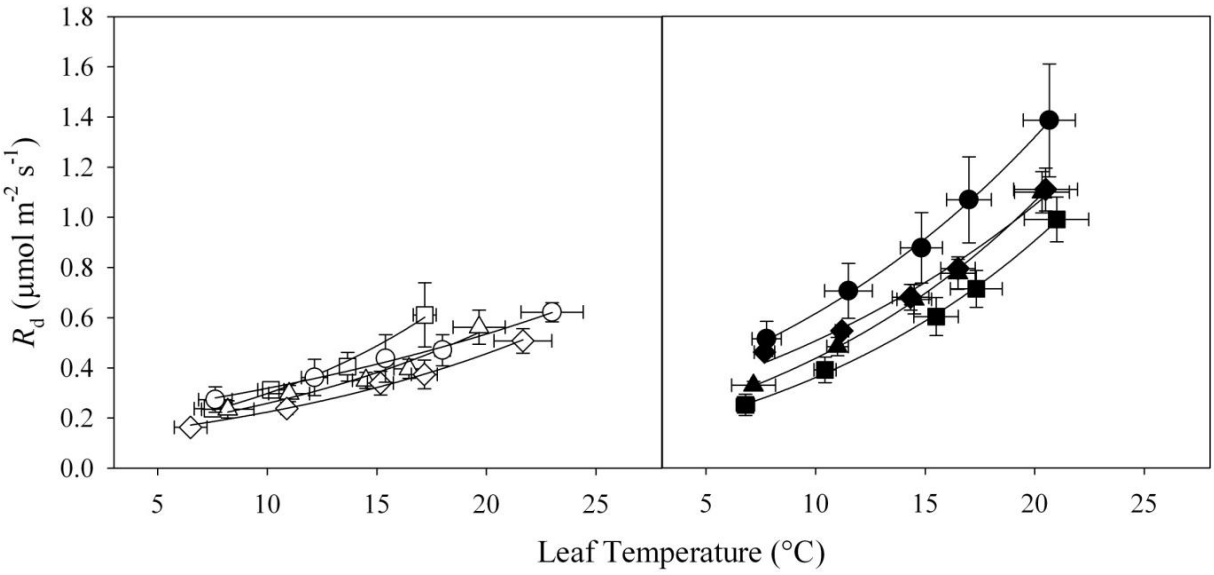

Figure 2.5 Response of respiration to temperature under varying soil water content for the grass (open symbols) and kānuka (closed symbols). The symbols indicate variations in soil water content i.e., 10 % (□), 20 % (Δ), 30 % (◊) and 40 % (○). Values shown are means (± standard error) where n = 6. The lines are fitted to the data using a modification of an Arrhenius function described by Equation 2.4.

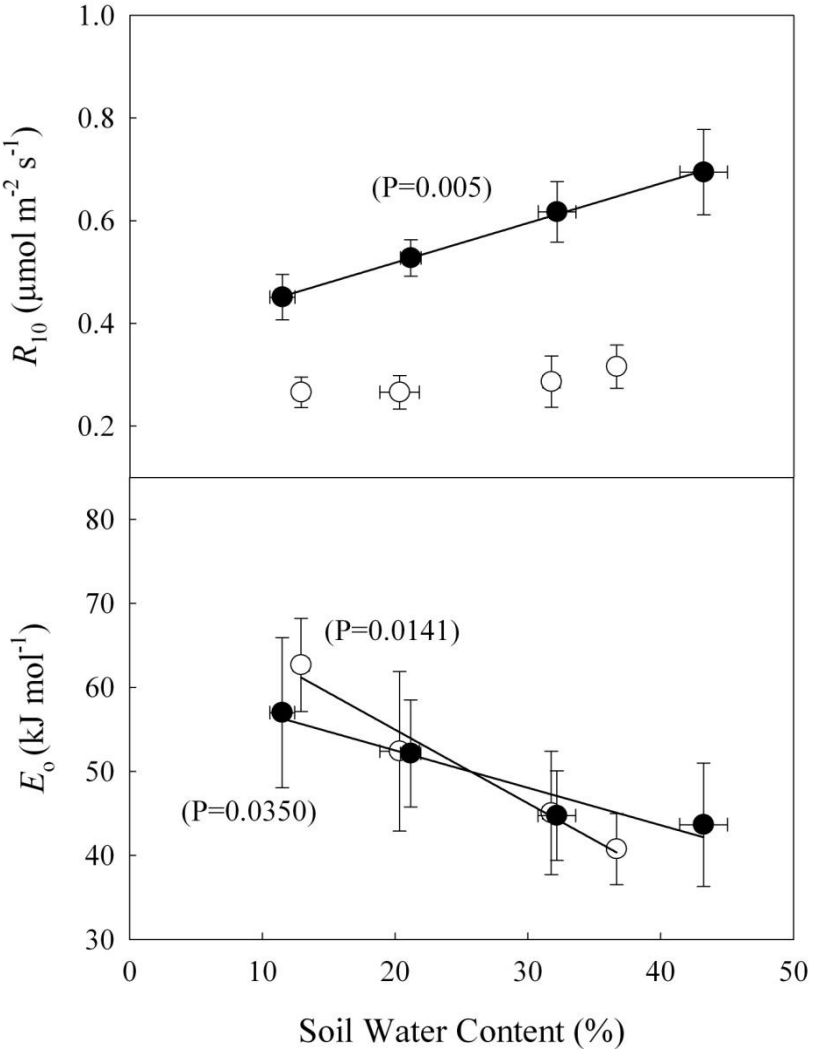

Figure 2.6 Response of dark respiration parameters calculated from fitted temperature response curves in Figure 2.4 to varying soil water content for the grass (open circles) and kānuka (closed circles). The regression relationships between R_{10} and E_o in the grass and kānuka with varying soil water content are presented in Table 2.5.

Table 2.4 Parameters obtained by fitting respiration response data to the mixed effect model described by equation 2.6 using leaf temperature and soil water content as variables for the grass and kānuka.

Species	**Variables**	R_{10} (μmol m^{-2} s^{-1})	E_o (kJ mol^{-1})	θ_c (%)	**P value**
Grass	Leaf temperature	0.29	38.2		< 0.001
Kānuka	Leaf temperature, Soil water content	0.62	43.5	15	< 0.001

2.3.4 Balance between photosynthesis and respiration

The ratio of dark respiration to photosynthesis (R/A_{sat}) varied with varying soil water content for both the grass and kānuka (Figure 2.7). The R/A_{sat} ratio was higher at low and high soil water content conditions whereas it remained low at moderate soil water content.

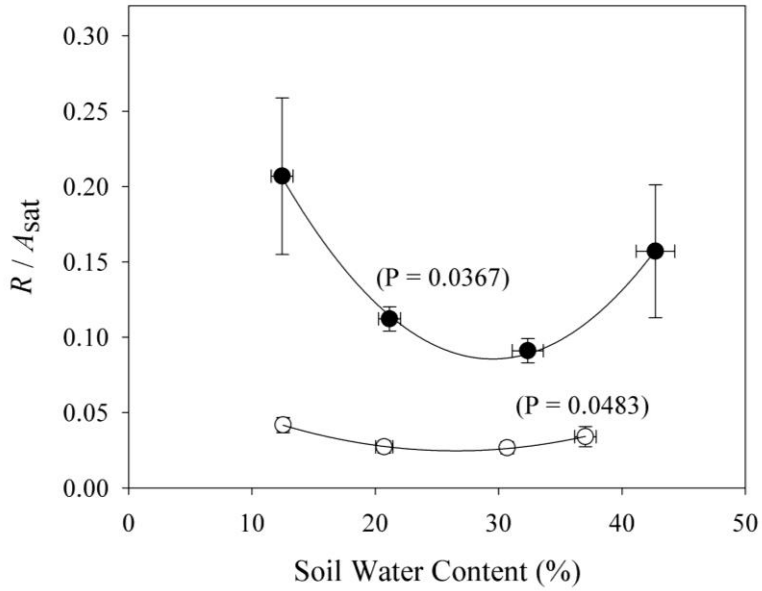

Figure 2.7 The ratio between foliar respiration (at a night temperature of 10 °C) and net photosynthesis during day (at 20°C) as a function of soil water content in the grass (open circles) and kānuka (closed circles). The regression parameters fitting the relationships are presented in Table 2.5.

Table 2.5 Summary of regression statistics for significant relationships ($P < 0.05$) between respiratory parameters and soil water content (independent variable) for the grass and kānuka.

Figure	Regression relationship	r^2
	Grass	
2.6	E_o (kJ mol^{-1}) = 72.4963 - 0.8761 θ (%)	0.97
2.7	R / A_{sat} = 0.000087 θ^2 - 0.0046 θ (%) + 0.0854	0.99
Figure	**Regression relationship**	**r^2**
	Kānuka	
2.6	R_{10} (µmol m^{-2} s^{-1}) = 0.3639 + 0.0077 θ (%)	0.99
2.6	E_o (kJ mol^{-1}) = 61.4065 - 0.4449 θ (%)	0.93
2.7	R / A_{sat} = 0.0004 θ^2 - 0.0244 θ (%) + 0.4458	0.99

2.4 DISCUSSION

Many studies have investigated the influence of temperature or soil water content on changes in photosynthesis and respiration. However, few studies have examined the combined influence of both these environmental drivers on both photosynthesis and respiration. The findings of the present study are therefore important in contributing to an improved understanding of the response of photosynthetic and respiratory parameters in grass and a shrub to varying soil water content and temperature.

2.4.1 Photosynthetic response to changes in growth temperature and soil water content

The results of this experiment demonstrated that the temperature response of photosynthetic parameters in both the grass and kānuka varied significantly with soil water content during a drying cycle. These responses were independent of nitrogen and phosphorus content, as these values did not change during the experiment. Changes in photosynthesis, including a reduction in capacity of RuBP carboxylation and RuBP regeneration, were clearly evident in both the grass and kānuka in response to declining soil water content. This finding corroborates recent work reporting reduction in photosynthesis in relation to water stress (Tezara *et al.* 1999; Lawlor and Cornic 2002; Turnbull *et al.* 2002b; Lawlor 2002b; Flexas *et al.* 2004).

Moreover, changes in leaf temperature from 8 to 25 °C also resulted in a significant change in the balance between RuBP regeneration and carboxylation rate and there was a strong and negative relationship between the leaf temperature and the J_{max}/V_{cmax} ratio. The temperature effect on V_{cmax} and J_{max} was consistent with that reported in previous studies (Walcroft *et al.* 1997; Medlyn *et al.* 2002b; Dungan *et al.* 2003; Diaz-Espejo *et al.* 2006; Miyazawa and Kikuzawa 2006; Silim *et al.* 2010). The values of V_{cmax} and J_{max} observed for kānuka and the grass at 20 °C were comparable with the values of V_{cmax} and J_{max} observed in previous studies under similar conditions for different C_3 grasses (e.g., *Lolium perenne*, Moon 1990; Wohlfahrt *et*

al. 1999) and shrubs (e.g., *Dodonaea angustissima*, Schulze *et al.* 1982). Whitehead *et al.* (2004b) reported the values of V_{cmax} and J_{max} of field grown *K. ericoides* (44.9 ±2.9 and 88.9 ±5.5 μmol$m^{-2}s^{-1}$, respectively, at 20 ºC leaf temperature), and the values estimated in this study are similar. The J_{max}/V_{cmax} ratios in this study at 20 ºC when soil water content was above 30 % were also close to the values previously observed by Whitehead *et al.* (2004b): 2.32 ± 0.08 for young kānuka plants grown under nursery conditions. Since these photosynthetic parameters do not have the same temperature dependence, the ratio of J_{max}/V_{cmax} may vary with temperature (Farquhar and von Caemmerer 1982; Hikosaka *et al.* 1999; Onoda *et al.* 2005a). Hikosaka *et al.* (2006) illustrated that the optimal temperature of photosynthesis shifted with alteration in the J_{max}/V_{cmax} ratio. The decrease in the J_{max}/V_{cmax} ratio at higher temperatures for both the grass and kānuka clearly illustrates the higher temperature sensitivity for V_{cmax} than for J_{max}. Similar changes in the J_{max}/V_{cmax} ratio have been reported previously (Walcroft *et al.* 1997; Medlyn *et al.* 2002b; Yamori *et al.* 2005; Way and Sage 2008a). This trend in both species may indicate an acclimation response and has been reported to be the effect of greater activation energy for V_{cmax} than for J_{max} (Dreyer *et al.* 2001; Onoda *et al.* 2005a). Previous studies have indicated that variation in the J_{max}/V_{cmax} value may be associated with differences in the allocation of leaf nitrogen or photosynthetic proteins to the carboxylation and electron transport processes at varying temperatures (Hikosaka *et al.* 1999; Onoda *et al.* 2005b; Yamori *et al.* 2005).

Importantly, the sensitivity of photosynthetic parameters also varied with soil water content in both species. The net photosynthesis at saturating irradiance measured at 20 ºC ($A_{sat,20}$) displayed a curvilinear response to soil water content, with very low values at low soil water content and higher values at moderate soil water content. Similarly, Ambebe and Dang (2009; 2010) found that low soil water content could influence net photosynthesis and biomass production in white birch seedlings by inhibiting the enhanced effect of increasing soil temperatures. The optimum soil water content for $A_{sat,20}$ was around 30 % for both the plant

types. Furthermore, results of the present study showed that $V_{cmax,20}$ and $J_{max,20}$ increased with increasing soil water content, in both the grass and kānuka. This finding supports previous studies showing that at low relative water content, RuBP synthesis of leaves decreases (Tezara *et al.* 1999; Lawlor 2002b; Grassi and Magnani 2005; Limousin *et al.* 2010). The response of photosynthetic parameters (A_{sat}, V_{cmax} and J_{max}) of both kānuka and the grass to varying soil water content is comparable with the results of a soil water content related study on photosynthetic parameters of the tree species *Quercus mongolica* (Wei *et al.* 2008). However, at higher soil water content $V_{cmax,20}$ and $J_{max,20}$ values continued increasing while $A_{sat,20}$ declined above 30 % soil water content in both the grass and kānuka, indicating that photosynthetic capacity is not solely regulated by these metabolic parameters. Although soil water content in the range from 10 to 40 % affected RuBP regeneration and carboxylation rates, changes in the ratio of $J_{max,20}$ /$V_{cmax,20}$ were much more limited. Clearly, this balance is very tightly co-regulated with variation in soil water content. This finding has direct implications for efforts to improve models of canopy carbon exchange by improving temperature sensitivity of photosynthetic parameters under fluctuating soil water content.

It has been commonly reported that the activation energy of V_{cmax} (H_{av}) is positively associated with growth temperatures (Yamori *et al.* 2005; Hikosaka *et al.* 2006; Bauerle *et al.* 2007; Kositsup *et al.* 2009) and the resulting photosynthetic performance is largely associated with the Rubisco activation state and Rubisco kinetics (Yamori *et al.* 2006; Weston *et al.* 2007; Hozain *et al.* 2010). However, contrasting responses in the temperature sensitivities of V_{cmax} with increasing growth temperature have also been reported (Medlyn *et al.* 2002b; Kattge and Knorr 2007; Warren 2008; Dillaway and Kruger 2010). Moreover, at low soil water content the activation state of Rubisco is reduced (Parry *et al.* 2002), which may in turn inhibit photosynthesis, especially at extreme conditions (Bota *et al.* 2004). Therefore, under such conditions, homeostasis in photosynthesis is disturbed due to an imbalance between electron

transport (NADPH and ATP synthesis) and the dark reactions of CO_2 assimilation affecting the regeneration of RuBP (Tezara *et al.* 1999; Lawlor 2009). In the present study the activation energy of V_{cmax} (H_{av}) and J_{max} (H_{aj}) increased with soil water levels, demonstrating a non-linear response. Under moderate to high soil water levels, the H_{av} and H_{aj} values for the grass fell within the range of values observed in several C_3 grass species by Wohlfahrt *et al.* (1999) while the values of H_{av} and H_{aj} for kānuka were close to the values observed in the mediterranean sclerophyll shrubs (Tenhunen *et al.* 1990). Therefore, it is clear from the present study that soil water content can influence the photosynthetic response to temperature and suggests the relevance of considering soil water content in improving modelling of temperature responses.

Various studies have found strong stomatal limitation of photosynthesis (Escalona *et al.* 1999; Flexas and Medrano 2002; Ghannoum *et al.* 2003; Flexas *et al.* 2004; Tissue *et al.* 2005). In a study correlating photosynthetic capacity with stomatal conductance, Wong *et al.* (1979) identified a relationship between stomatal conductance (g_s) and the rate of CO_2 assimilation (A) and recognized a decline in both g_s and A due to water stress. Further studies on the influence of water stress on stomatal behaviour have observed a progressive reduction in C_i with increasing water stress (Lawlor 1995; Flexas and Medrano 2002). In the present study, the direct impact of soil water content on relative stomatal limitation of photosynthesis was not strong (a moderate increase in relative stomatal limitation at low soil water content in the grass but no increase in kānuka). Thus we must look to other mechanisms linking changes in soil water content to the thermal sensitivity of photosynthetic parameters in these species.

2.4.2 Respiratory response to changes in growth temperature and soil water content

The thermal response of respiration in the grass and kānuka varied significantly with varying soil water content. Results showed a positive relationship between the respiration rate and soil water content, resulting in decreased R_{10} values with decreasing soil water content for

both the species. This result is consistent with previous studies showing that water-stress induces lower rates of respiration (Collier and Cummins 1996; Flexas *et al.* 2005; Flexas *et al.* 2006; Galmés *et al.* 2007; Atkin and Macherel 2009; Vassileva *et al.* 2009; Vassileva *et al.* 2011). It is apparent from the current study that the temperature sensitivity of respiration under different soil water content varies with respect to species. For the grass, R_{10} was not strongly sensitive to soil water content, indicating relatively high tolerance level to varying soil water contents. The upward shift in R_{10} with increasing soil water content was, by comparison, higher in kānuka and the data analysis using non-linear mixed effect models revealed a significant reduction in R_{10} when soil water content was less than 15 %. The shift in R_{10} values is analogous to the 'Type II' thermal acclimation, which is a temperature-mediated change in respiratory capacity (Atkin and Tjoelker 2003). The reduced rate of respiration with declining soil water content is unlikely to be a substrate limited mechanism because changes in the content of sugar, starch, nitrogen and phosphorus were not identified in both species. The consistency in increasing soluble carbohydrate content with decreasing soil water content regardless of the significant reduction in photosynthesis is in agreement with the findings of previous studies (Ghashghaie *et al.* 2001; Slot *et al.* 2008). The impact of water stress on photosynthesis is reported to be much faster and more marked than the effect on respiration (Flexas *et al.* 2005; Slot *et al.* 2008), which exacerbates with severity (Ribas-Carbo *et al.* 2005) and reduces growth (Flexas *et al.* 2006) and maintenance (which require respiratory ATP) respiration (Hoefnagel *et al.* 1998). The temperature dependent changes in growth and maintenance respiration and ion uptake can consequently alter the demand for ATP (Atkin *et al.* 2005a). In the present study, the decrease in soil water content not only resulted in a decrease in basal respiration but also changed the temperature sensitivity of respiration in both the plant types. The downward adjustment of the rate of leaf respiration at low soil water content would possibly maintain the balance of net carbon exchange within a developing shrubland undergoing seasonal changes over a year.

Unlike R_{10} values, E_o values were similar in both the grass and kānuka. E_o was higher at low soil water contents and declined linearly with increasing soil water content in both the grass and kānuka. This indicates that respiration is more sensitive to temperature at lower soil water content. Previous studies aiming the response of plant respiration to water stress recognised reduction in the energy-conserving, cyanide-sensitive, cytochrome respiration rate with a concomitant increase in the energy-wasting, cyanide-resistant, alternative respiration rate (Ribas-Carbo *et al.* 2005; Vassileva *et al.* 2009; Vassileva *et al.* 2011) causing mitochondrial electron shift. Under water-stressed condition, this biochemical regulation may have a critical role in maintaining the balance of mitochondrial electron partitioning owing to their relative variation in ATP generation. It seems that the activated alternative pathway is leading the major role in contributing the electron flow to maintain respiration and to prevent over reduction of ubiquinone pool (Armstrong *et al.* 2008) that would possibly make higher E_o values under water stressed conditions. Vassileva *et al.* (Vassileva *et al.* 2011) observed a higher temperature response of respiration in three winter wheat varieties exposed to drought stress in connection with reduced respiration rate. The higher demand for respiratory energy at low soil water content is likely associated with water stress induced higher demand for ATP or cellular maintenance (e.g. repairing degraded proteins, improving membrane transport and retaining ion gradients) and may also be linked to photosynthetic acclimation (Atkin *et al.* 2000b; Atkin and Macherel 2009). Therefore, higher E_o values are likely linked to the potentially higher fraction of respiration occurring via alternative pathway at low soil water levels. Armstrong *et al.* (2008) observed a higher temperature sensitivity of alternative oxidase pathway to short term changes than that of cytocrome pathway in *Arabidopsis thaliana.* The water stress induced reduction in cytochrome activity consequently affects phosphorylation efficiency and could result in lower ATP yield while increasing alternative pathway could possibly increase the ATP yield as it is more sensitive to temperature than cytochrome oxidase. But the influence of soil water content on

temperature sensitivity of these two respiratory components is unclear and their relative roles in electron partitioning under water-stressed conditions need to be explored.

Nevertheless, Turnbull *et al.* (2001) reported contrasting results in the response of respiration to temperature in deciduous trees at sites with contrasting soil water availability. There is growing evidence that seasonal changes in environment can alter the leaf traits (e.g. specific leaf area, leaf nitrogen, soluble carbohydrate) that would appreciably influence the temperature sensitivity of plant respiration (Atkin *et al.* 2000b; Turnbull *et al.* 2003). At low temperatures, respiration is limited by enzyme capacity where as at higher temperature it is likely to be regulated by the availability of substrates or enzyme capacity. Therefore, changes in the temperature sensitivity of respiration (Q_{10} or E_o) in response to soil water availability may likely vary with respect to leaf metabolic state under field conditions. Although the changes in E_o under varying soil water levels highlight the importance of considering the effect of soil water content on the temperature response of respiration, a detailed investigation of seasonal and site-specific variation in the temperature response of leaf respiration will further increase the confidence in incorporating the dynamic response of E_o to changing soil water content.

2.4.3 Balance between foliar respiration and photosynthetic capacity

In this experiment, the photosynthetic and respiratory rates of the grass and kānuka were positively correlated with the soil water content. Similar findings were reported by Turnbull *et al.* (2001) on deciduous tree species at contrasting soil water content. R/A_{sat} permits the assessment of the balance between the respiratory cost and photosynthetic capacity under varying soil water content at the leaf level. R/A_{sat} values were found to be relatively high at low and high soil water availability in both plant types. Previous studies have reported a higher R/A_{sat} ratio under drought (2005; Flexas *et al.* 2006; Atkin and Macherel 2009). The R/A_{sat} ratios varied between the species and were found to be higher in kānuka compared to the grass. The variation

in R/A_{sat} ratio is due to the changes in photosynthetic (curvilinear) and respiratory (linear) responses of the grass and kānuka to varying soil water content. The impact of soil water content on leaf metabolic state altered the temperature sensitivity of photosynthesis and dark respiration, affecting carbon fixation. Therefore, complete homeostasis of R/A_{sat} is not achieved at varying soil water content owing to the differences in the temperature sensitivity of these processes.

Various climate warming experiments investigating plant responses to increasing temperature reported species-specific changes in above-ground biomass accumulation with changing soil water content (Harte and Shaw 1995; Loik and Harte 1996; 1997; Loik *et al.* 2000). Results of Loik *et al.* (2000) showed that if soil water content is limited due to warming, increasing leaf temperature had limited effect on leaf photosynthesis when compared to the effect of differences in soil water content which cosequently lowered plant water potential resulting reduction in above-ground biomass accummulation. This is associated with differential leaf thermal tolerance that may likely alter plant growth stages during the entire growing season, affecting carbon fixation (e.g. Zhou *et al.* 2007). Therefore, production of photosynthates will determine whether or not a positive carbon balance can be maintained and to keep a positive carbon balance under adverse conditions (eg. drought); complete recovery of stress condition is essential (Slot *et al.* 2008). In addition, acclimation potential for photosynthesis and respiration may re-establish a new balance between photosynthesis and respiration rate in response to changes in temperature, but this is not universal (Zhou *et al.* 2007) because the temperature sensitivity of respiration is different from that of photosynthesis (Gifford 2003; Atkin *et al.* 2006b). But in many species, homeostasis of the ratio of respiration to photosynthesis is attained as a result of thermal acclimation (Loveys *et al.* 2003; Atkin *et al.* 2005a; Atkin *et al.* 2006a; Atkin *et al.* 2006b). The response in this study shows that the rate of increase in photosynthesis with increasing soil water content is greater than that of respiration which results in a progressively greater apparent carbon gain at moderate soil water content. However, at lower and

higher soil water content conditions the assimilation of carbon is limited and a relatively higher proportion of carbon is respired by these plants under stress, indicating relatively more negative net carbon balance. The findings clearly show that there is a significant uncertainty in results from large-scale carbon balance models that presume consistency of the R/A_{sat} ratio under all soil water content conditions.

2.5 SUMMARY AND CONCLUSIONS

In conclusion, soil water content has a greater effect upon the short-term temperature response of photosynthesis than that of respiration for plants growing in controlled conditions. Soil water deficit induced an inhibition of photosynthetic capacity of the leaves due to a reduction in carboxylation efficiency and RuBP regeneration capacity in both plant types. It is clear that the combined impacts of soil water availability and temperature are highly species-specific and so predicting the effects of temperature and soil water content on respiration and photosynthesis requires knowledge of the individual responses of these physiological processes. A better understanding of these processes is therefore required to support the wider use of interactions and effects of soil water content and temperature on photosynthesis and respiration. Currently, carbon balance models incorporate either temperature effects (e.g. on photosynthetic or respiratory responses) or direct soil water effects (e.g. on stomatal behaviour) on net carbon exchange. However, there is less attention to changes in the temperature sensitivity of carbon exchange parameter in response to changes in soil water content. Since the temperature sensitivity of photosynthetic and respiratory parameters are influenced by soil water content, it is important to develop ways to incorporate changes in their biochemical activity while modeling canopy carbon exchange and water balance. Moreover, significant differences observed in leaf-level respiration rates of the grass and kānuka at varying soil water content may have important mechanistic implications in terrestrial carbon-cycle models for understanding and predicting the

rate of carbon exchange in a grassland or shrubland ecosystem, experiencing drought in future as a result of ongoing climate change. However, further investigation of seasonal variation in the temperature responses of respiration in these plant types under field conditions are essential for modelling leaf-level to ecosystem-level responses.

2.6 DIRECTIONS FOR FUTURE WORK

In this chapter, the effects of temperature and soil water content on photosynthesis and respiration of a grass and a native shrub were investigated under controlled growth cabinet conditions. This experiment showed that the net carbon exchange is highly sensitive to these environmental drivers. In order to understand the extent of photosynthetic and respiratory acclimation potential of these plants to varying environmental conditions, improved understanding of the effect of seasonal variation in temperature and soil water content on these physiological processes under their natural habitat is essential. Furthermore, investigation of the interactive effects of temperature and soil water content on the relationship between photosynthesis and respiration in field conditions will improve modelling capabilities to predict the effects of changing climate on the long-term carbon balance of the developing shrubland ecosystem. The next chapter focuses on the effects of diurnal and seasonal fluctuations in temperature and soil water content on the net carbon exchange for the grass and kānuka growing at a field site near Oxford in North Canterbury, New Zealand. The effects of acclimation of photosynthesis and respiration to environmental variables are also considered.

CHAPTER 3

Assessing the impact of seasonal changes in temperature on foliar carbon exchange of the shrub, kānuka (*Kunzea ericoides* var. *ericoides*) established in the grass, Yorkshire fog (*Holcus lanatus* L.) at a field site.

Chapter 3

3.1 INTRODUCTION

Predicting the response of ecosystem carbon exchange to environmental perturbations is a major challenge for the scientific community. According to the IPCC (IPCC 2007), climate change will alter the structure and dynamics of various ecosystems. Within terrestrial ecosystems, the most important processes that influence carbon exchange and storage in plants are photosynthesis (carbon acquisition) and respiration (carbon release) (Ryan 1991; Hunt *et al.* 2004). A fine balance between these two processes regulates the net carbon balance of a terrestrial ecosystem. These processes are not only inter-dependent through carbohydrate status and the demands for ATP (Atkin *et al.* 2006b), but they are also highly dependent on climatic and environmental perturbations such as changes in temperature (Berry and Bjorkman 1980; Sage and Kubien 2007), soil water deficit (Yordanov *et al.* 2000; Lawlor and Cornic 2002), light availability (Wang and Jarvis 1990; Araya *et al.* 2008) and nutrient availability (Evans 1989; Reich *et al.* 1998; Whitehead *et al.* 2004b; Carswell *et al.* 2005).

To fully understand the response of photosynthesis and respiration to changing climatic and environmental factors, it is important to understand how photosynthesis and respiration respond to temperature over the short and long-term. The temperature sensitivities of photosynthesis (Hikosaka *et al.* 1999; Medlyn *et al.* 2002a) and respiration (Atkin *et al.* 2005b; Ow *et al.* 2010) vary among species. The optimum temperature for photosynthesis for C_3 plants generally has optima at temperatures between 20 and 30 ^{o}C (Larcher 2003). However, the instantaneous temperature response of plant respiration is different from that of photosynthesis – and increases exponentially with temperature until the rate decreases rapidly near the lethal heat limit (Körner 2007). Following the biochemical model of Farquhar *et al.* (1980), photosynthetic rates are limited by RuBP (ribulose – 1,5 bisphosphate) carboxylation rate (V_{cmax}) and the rate of RuBP regeneration (J_{max}). Several studies have shown that changes in growth temperature not

only alter the relative values of these parameters but also influence their temperature dependence (Hikosaka *et al.* 1999; Onoda *et al.* 2005a).

Prolonged exposure of plants to a change in temperature may result in acclimation: the thermal optimum of photosynthesis may shift towards the new growth temperature, which is often accompanied by a change in the activation energy for V_{cmax} (Hikosaka *et al.* 2006). Acclimation results in an improved performance of the plants under the new growth conditions (Berry and Bjorkman 1980). Similarly, long-term changes in temperature may lead to respiratory acclimation *via* changes in the temperature sensitivity (E_o) and / or a shift in the basal respiratory capacity i.e., R_{10} (Atkin and Tjoelker 2003; Atkin *et al.* 2005a; Ow *et al.* 2008a). The term Q_{10} is the ratio of rates of respiration over a temperature change of 10 to 20 °C and E_0 describes the temperature sensitivity of respiration. However, Q_{10} and E_0 values are related. The acclimation process is often associated with temperature-mediated changes in the availability of substrates, and demand for respiratory energy (Atkin and Tjoelker 2003). The mechanisms behind the thermal acclimation of photosynthesis and respiration are not fully clear, but understanding these processes is critical for predicting the responses in the carbon balance of natural vegetation to global climate change.

Certain questions remain regarding thermal acclimation. The degree of photosynthetic and respiratory acclimation varies among species (Berry and Bjorkman 1980; Atkin *et al.* 2006b) from partial to full acclimation, resulting in changes in the respiration/photosynthesis ratio (Gifford 2003; Atkin *et al.* 2006b; Ow *et al.* 2010). It is unknown why photosynthesis and respiration acclimate to varying degrees, and why the degree of acclimation varies among plant groups and plants with different thermal histories. Another question is how quickly does acclimation occur? With regard to respiration, rapid (1-7 days) acclimation to changing temperatures has been reported (Bolstad *et al.* 2003; Armstrong *et al.* 2008), although full acclimation has been found to require weeks to months of adjustment to a new temperature in

some species (Ow *et al.* 2008a; Ow *et al.* 2008b; Searle *et al.* 2010). Photosynthesis has been found to acclimate generally more slowly and to a lesser extent than respiration (Lambers *et al.* 2008; Ow *et al.* 2008a; Ow *et al.* 2008b). Thus, generalisations cannot be made about the timing or degree of thermal acclimation in all plant groups.

Just as temperature has a significant effect on metabolic processes, soil water content is also an important environmental variable that influences photosynthesis and respiration and consequently affects the growth and production of plants (Chaves *et al.* 2003). Soil water deficit results in a decrease in photosynthesis in plants due to stomatal and / or non-stomatal limitations i.e, *via* changes in biochemical capacity (Lawlor 1995; Escalona *et al.* 1999; Panković *et al.* 1999; Tezara *et al.* 1999; Subrahmanyam *et al.* 2006). Two studies investigating the effect of water deficit on photosynthesis in grapevines demonstrated that moderate irrigation is ideal for maintaining high photosystem II efficiency and preventing photochemical down-regulation (Flexas *et al.* 1998; Flexas *et al.* 1999). The impact of low soil water availability on plant respiration is less pronounced than that of photosynthesis (Atkin and Macherel 2009). Respiration has been shown to decrease under water deficit due to a decline in enzyme activity and mitochondrial protein concentration (Atkin and Macherel 2009). The impact of water deficit on the carbon balance of plants depends on the relative effect on respiration and photosynthesis and the degree and timing of acclimation for each process (Flexas *et al.* 2006).

Although many studies have investigated the response of photosynthesis and respiration to temperature, relatively few studies have investigated the combined effect of varying soil water content and temperatures experienced by the plants diurnally and seasonally under natural conditions (Ogle and Reynolds 2002). Since regenerating shrubland has been considered as a potential sink for offsetting atmospheric carbon emissions by storage in biomass, it is important to determine the rate of carbon uptake and storage and the environmental and site related factors regulating carbon sequestration. In this study, I investigated the photosynthetic and respiratory

responses of a grass and a shrub in field conditions to seasonal changes in soil water content and air temperature. The system was in the early stage of reversion from pasture to shrubland. The major objectives of this study were to: 1) investigate the parameters needed for modelling net carbon exchange by determining the response of photosynthetic and respiratory components to driving variables; 2) determine the temperature response of leaf-scale rates of respiration and the extent to which this response changes with environment; and 3) investigate the relationship between these gas exchange processes and foliage characteristics. This understanding will inform a simulation of plant productivity through modeling the net carbon balance of a developing shrubland in response to seasonal changes in temperature and soil water content in New Zealand (Chapter 4).

3.2 MATERIALS AND METHODS

3.2.1 Site description

This study was carried out at an experimental site near Oxford, New Zealand (latitude 43.3 ºS, longitude 172.2 ºE), elevation 34 m above sea level. The study area was a 1 ha grazed grassland that has been converted to a shrubland by planting nursery grown native kānuka (*Kunzea ericoides* var. *ericoides*) shrub seedlings in a grid at a spacing of 1 m (Figure 3.1). Grass species in this area were predominantly Yorkshire fog (*Holcus lanatus* L.), brown-top (*Agrostis capillaris* L.) and perennial ryegrass (*Lolium perenne* L.). The terrain was nearly flat and the soil at this site was poorly drained Taitapu Typic Orthic Gley (New Zealand Soil Classification) (Hewitt 1998), with a perched water table. Kānuka height ranged between 500 mm and 1500 mm while the grass height ranged between 100 mm and 750 mm. Figure 3.1 shows the experimental site, while Figure 3.2 shows typical seasonal changes in community structure at this site. A weather station was set up at the experimental site (Figure 3.1).

Figure 3.1 The experimental site in Oxford, New Zealand. Kānuka seedlings are developing on pasture and the weather station is visible.

Measured variables include air temperature and relative humidity (Vaisala HMP50, Helsinki, Finland), photosynthetically active radiation (Q) (LI-190, LICOR, Lincoln, NE, USA) and volumetric soil water content (θ) (Model ML2x ThetaProbe, Delta-T Devices, Cambridge, UK). Weather data are shown in Figure 3.3. All data were sampled at three-second intervals and averaged every 30 minutes.

The measurements were made during the period November 2007 to October 2008. Volumetric soil water content was measured at three different depths (50, 100 and 300 mm) and daily average values were collected using permanently installed and logged soil moisture sensor (Model ML2x, HH2 ThetaProbe, Delta-T, Cambridge, UK) under varying depths in the field. In addition, root-zone soil water content measurements of the sampling plants were recorded by inserting a hand held soil moisture sensor (Model ML2x, HH2 ThetaProbe, Delta-T, Cambridge, UK) about 50-60 mm away from the base of the sampling plants.

Figure 3.2 Typical seasonal changes in community structure observed in the study area

3.2.2 Gas exchange measurements and tissue analysis

Photosynthesis and respiration were measured on fully expanded leaves of randomly selected kānuka and grass using two cross-calibrated, portable open-flow gas analysis systems with CO_2 control (Model LI-6400, Li-Cor BioSciences, Lincoln, NE, USA). Gas exchange measurements were made with the standard 20 x 30 mm chamber. For kānuka, fully mature leaves growing on the secondary branches were selected (n = 6-10) while for the grass, fully expanded, mature leaves (i.e., the second and /or third leaf from the shoot apex) of the predominant grass species at the site (i.e., Yorkshire Fog) (n = 6 -10) were selected for each

measurement. Environmental conditions within the leaf chamber were maintained to match the ambient conditions at the site, unless otherwise specified.

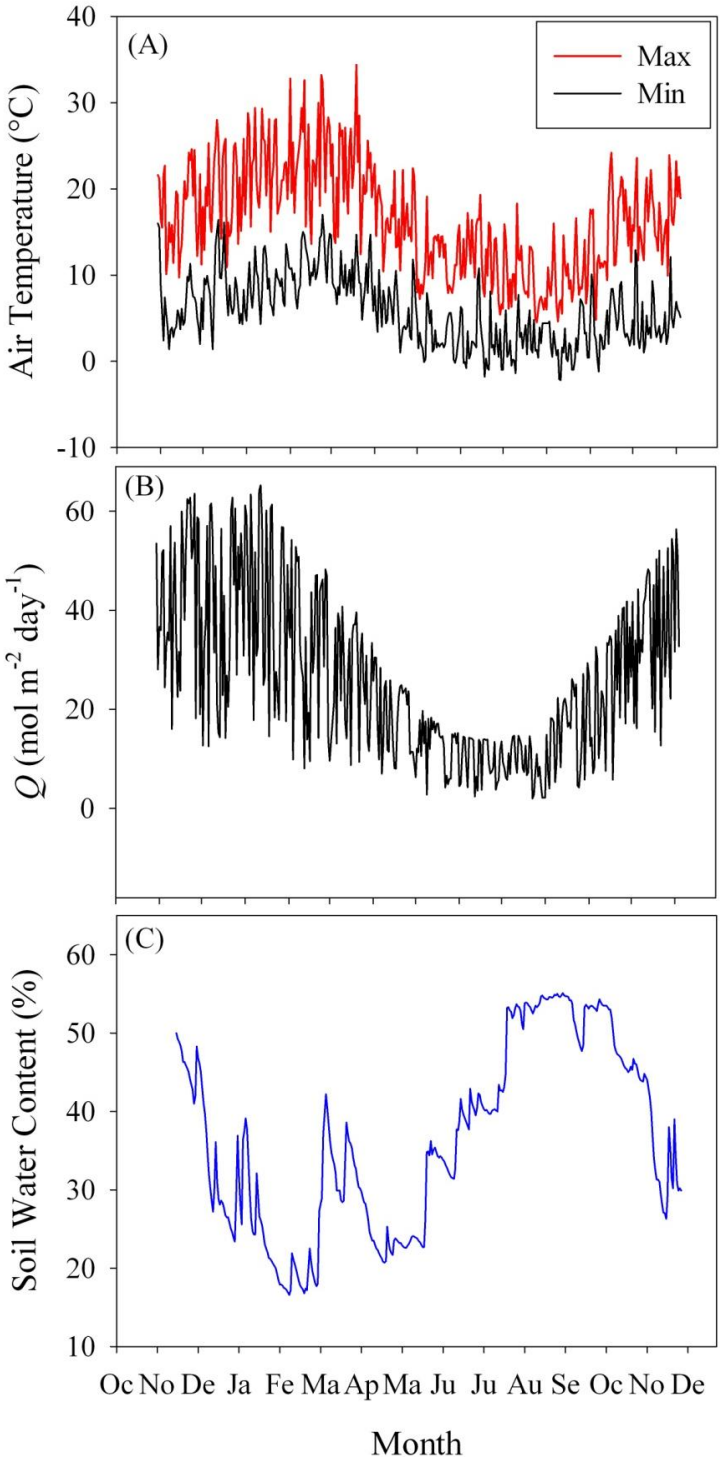

Figure 3.3 Seasonal variations in daily ambient temperature (minimum and maximum air temperature), photosynthetically active irradiance (Q) and soil water content (θ) at the Oxford experimental site in New Zealand (from November 2007 to October 2008).

Measurements of the response of photosynthesis (A) to intercellular CO_2 partial pressure (C_i) (A/C_i curves) were made for each sample by changing the external CO_2 concentration (C_a) in 15 steps (from 120 to 0 Pa) at a constant irradiance (Q) of 1500 µmol m^{-2} s^{-1}. Measurements were recorded when the photosynthetic gas exchange had equilibrated (taken to be when the coefficient of variation for the CO_2 concentration differential between the sample and reference analysers was below 1 % and visibly stable) at each C_a set point. This condition was typically achieved within 60 to 90 seconds after a stable set point had been reached. Later, light response curves were generated on the same leaves by varying the incident Q in 12 steps from 1500 to 0 µmol m^{-2} s^{-1} using a light source consisting of blue-red light-emitting diodes mounted on the top portion of the cuvette. During the light response curves, C_a was maintained at the ambient (37 Pa) concentration. The leaf temperature for the measurement of photosynthesis (set at the forecast maximum temperature for the day of measurement) was maintained using thermoelectric coolers. Vapour pressure deficit was maintained around 1.0 to 1.5 kPa within the cuvette. Leaf surface areas are presented on a one-sided basis for both plant types. Analysing high-resolution photographs of the leaves spread on a calibrated surface using Image-Pro Plus 7.0 software calculate surface area of the leaves within the cuvette. Leaf samples were dried for 48 hours at 70 ºC.

A/C_i response curves were used to determine values for the maximum rate of carboxylation (V_{cmax}), the apparent maximum rate of electron transport at saturating irradiance (J_{max}) and values of the limitation imposed by the stomata on the rate of photosynthesis (L_{stom}). The A/C_i response data were analysed using the biochemical model of photosynthesis as described by Farquhar, Caemmerer *et al.* (1980) using Sigma Plot, Software version 8.0 SPSS Inc. Chicago, Illinois. The rate of assimilation limited by carboxylation (A_c) and electron transport (A_q) is estimated by using the model equations of Farquhar *et al.* (1980) and it is shown in Chapter 2 (Equation 2.1 & 2.2).

The temperature dependence of leaf photosynthesis over the course of the year was determined considering the ambient temperature range experienced by leaves at an interval of about 30-40 days within the field, using the temperature response function of Leuning (2002) and it is exemplified in Chapter 2 (Equation 2.3).

The response of photosynthesis (A) to irradiance (Q) was used to determine values of maximum photosynthesis (A_{max}) at saturating irradiance and at ambient CO_2 concentration. The A/Q response was described by a rectangular hyperbola as described by Thornley and Johnson (2000). The relative limitation to photosynthesis imposed by stomatal and non-stomatal processes in the grass and kānuka was calculated from A/C_i curves by the method of Farquhar and Sharkey (1982).

Measurements of respiration on kānuka were made on the same leaves selected for measuring photosynthetic parameters. The branch was cut and and recut under water before transporting to the laboratory. Similarly, respiration measurements for the grass samples were made at the same laboratory on the second and / or third fully opened mature leaves of the sample. Grass samples were collected and transported from the same sampling area used for measuring photosynthesis. A grass tiller was gently dug up, with roots attached and wrapped in moist paper towel before transportation in a plastic bag. A number of previous measurements on plant respiration have shown that leaf respiration remains stable under these conditions for several hours (Turnbull *et al.* 2005; Ow *et al.* 2010). All the samples were placed in a dark growth cabinet with temperature control. Measurements of dark respiration were made at 8, 13, 18, 20 and 25 °C on these samples with the same gas analysis systems as were used for photosynthesis measurements. The growth chamber and leaf chamber were maintained at the same temperature. After changing to a new measurement temperature, leaves were allowed to equilibrate to the new temperature conditions for 30 minutes before measuring respiration. An average of five to six measurements was made over three minutes at an interval of 25 to 30

seconds at each temperature set-point. These were used to develop a temperature response curve of dark respiration following a previously described protocol (Turnbull *et al.* 2003). The respiration measurements on plant samples were completed within 4 to 5 hours after plant collection from the field. R_{growth} (actual rate of dark respiration at ambient minimum temperature) was estimated by considering the preceding 3-day minimum average temperature as the leaf temperature experienced by the plants.

The temperature response of respiration was analysed using a modified Arrhenius function as described by Turnbull *et al.* (2005) (refer chapter 2 - Equation 2.4). Standard non-linear curve fitting was performed using the Marquardt-Levenberg algorithm (Sigma Plot, v8.0 SPSS Inc. Chicago, Illinois).

The same leaf samples used for measuring photosynthesis and respiration were collected for measuring the surface leaf area. High-resolution photographs of leaf samples kept inside the leaf chamber were used to measure leaf area using Image pro Plus 4.5 software, Leeds Precision Instruments, Inc. Minneapolis. Leaves were then oven dried for 48 hours at 70 ºC. The mass of dried samples and leaf area were used to calculate the specific leaf area (*S*). Additional leaf samples collected from the field were also dried at 70 ^{o}C and ground in a ball-mill for measuring soluble sugar and starch content, following the methanol: chloroform: distilled water method of Tissue and Wright (1995). The level of sugar and starch content within the leaf samples was expressed on leaf area basis.

3.2.3 Model of response to temperature and soil water content

To understand the impact of environmental factors and to describe the response of photosynthetic and respiratory parameters to leaf temperature, T_l (K) and soil water content (θ), a non-linear mixed effect model (Pinheiro and Bates 2000) was used following the approach of Brown *et al.* (2009). The model included the temperature response functions of Leuning (2002)

for photosynthetic parameters and a modified Arrhenius function as described by Turnbull *et al.* (2005) for respiratory parameters, in addition to a linear function for the θ response as shown in chapter 2 (Equation 2.5 and 2.6) and the analysis was performed using R software, v2.11.1, R development Core Team (2010).

3.2.4 Statistical analysis

Repeated measures analysis of variance (ANOVA) was used to examine the effects of time on foliar properties, photosynthesis and respiration. Seasonal variations were considered significant if probabilities (P) were less than 0.05, using Minitab 15 (Minitab Inc. Pennsylvania, USA). Regression analysis was used to analyse the relationships between gas exchange characteristics (V_{cmax}, J_{max}, R_{10}, Q_{10}) and the environmental variables (including temperature and soil water content) as well as sugar and starch content in leaves on an area basis (Sigma Plot, Software version 8.0 SPSS Inc. Chicago, Illinois).

3.3 RESULTS

Maximum and minimum temperatures during the measurement period were within the range of 4.5 to 34.4 ^{o}C and –2.2 to 17 ^{o}C, respectively. The highest temperature occurred in late February and early March whilst the lowest temperature was observed in late July and early August. Similarly, annual precipitation at the site was approximately 1000 mm, most of which occurred during winter and spring (June to November). The lowest soil water content was recorded in January and the highest in August.

3.3.1 Leaf characteristics

Specific leaf area (*S*), starch and sugar content varied significantly ($P < 0.001$) in the grass and kānuka during the period of this experiment (Figure 3.4). *S* values were consistently higher in the grass than in kānuka, while starch and sugar content was consistently higher in

kānuka. The seasonal pattern of changes in *S*, measured from October 2007 to October 2008, was similar in both plant types, with relatively high *S* during summer to early autumn (January 2008 to April 2008) and low *S* during winter and early spring (July 2008 to October 2008 and October 2007 to November 2007). *S* varied between 21.1 to 29.2 $m^2 kg^{-1}$ and 6.3 to 8.8 $m^2 kg^{-1}$, for the grass and kānuka respectively (Figure 3.4A). Seasonal variation in starch content differed between the grass and kānuka. Starch content recorded for the grass varied between 0.39 g m^{-2} and 1.03 g m^{-2} with relatively high values in spring and low values in autumn. In kānuka, starch content varied between 1.42 g m^{-2} and 2.55 g m^{-2} with relatively high values in summer and low values in winter and spring (Figure 3.4B). Seasonal variation in sugar content was similar in kānuka and the grass, with higher levels in winter and spring and lower levels during the warmer months. Sugar content varied between 0.42 g m^{-2} and 1.14 g m^{-2} in the grass and between 1.13 g m^{-2} and 2.15 g m^{-2} in kānuka (Figure 3.4C).

3.3.2 Photosynthesis

Seasonal responses in photosynthesis of the field-grown grass and kānuka varied significantly ($P < 0.001$) over the period of the experiment (Figure 3.5). Values of V_{cmax} ranged from 42.3 to 191.8 µmol m^{-2} s^{-1} in the grass and from 14.9 to 84.5 µmol m^{-2} s^{-1} in kānuka (Figure 3.5A). The seasonal response of J_{max} (Figure 3.5B) was similar to that of V_{cmax} in both the plant types. The ratio of J_{max} to V_{cmax} varied between 1.2 and 3.2 in the grass and between 1.2 and 2.8 in kānuka (Figure 3.5C). Both the plant types exhibited a similar seasonal pattern in the ratio of J_{max} to V_{cmax}, which was found to be consistently high from autumn to spring and low in summer. A_{max} values varied between 10.4 and 21.6 µmol m^{-2} s^{-1} and between 3.5 and 28.0 µmol m^{-2} s^{-1}, in the grass and kānuka, respectively (Figure 3.5D). Relatively high values of A_{max} were observed from late winter to early summer for the grass, and they remained relatively constant during this period.

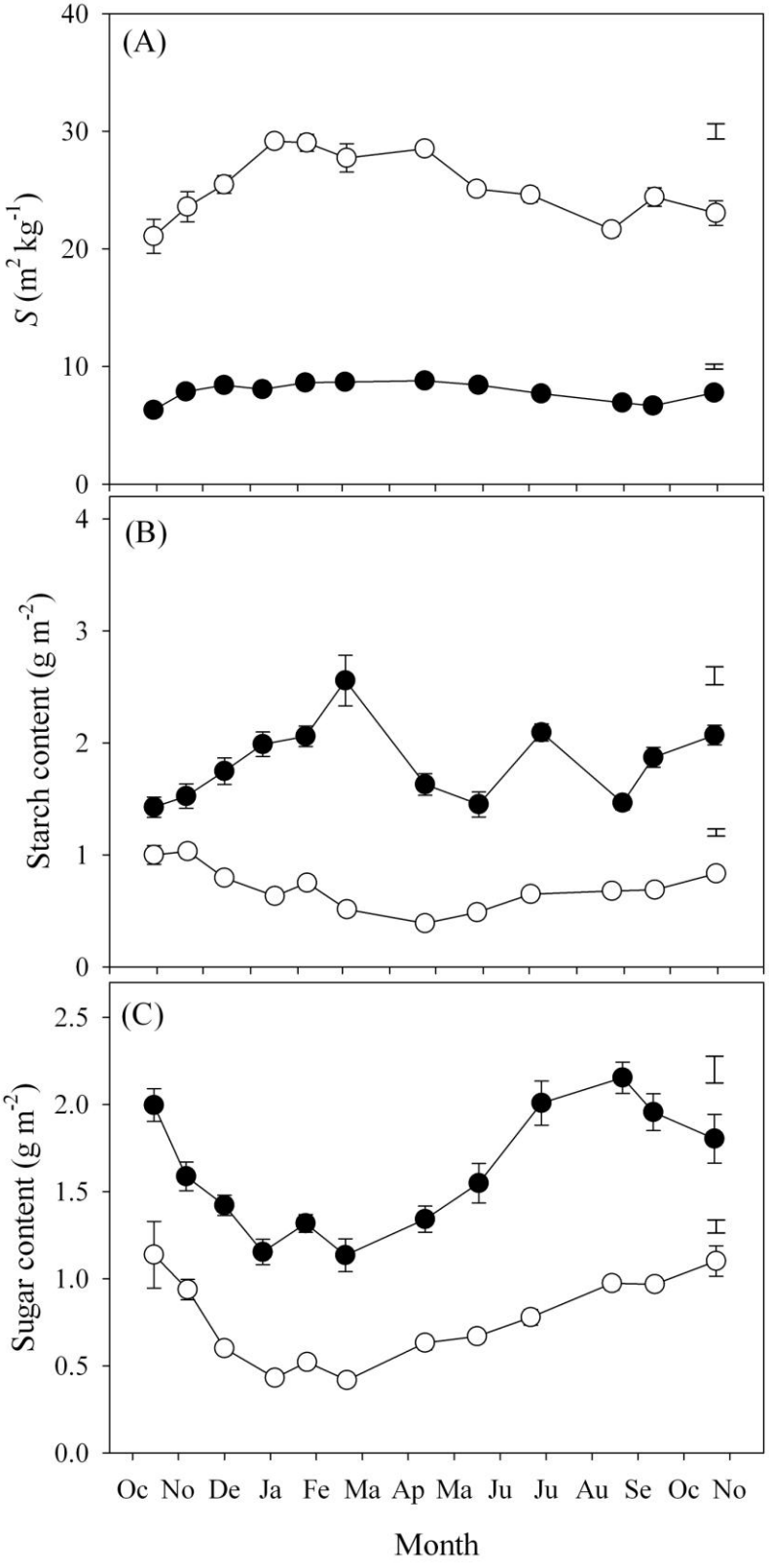

Figure 3.4 Seasonal variation in (A) specific leaf area, S, (B) starch and (C) soluble sugar content in field grown 2-3 year old kānuka (*Kunzea ericoides*) (closed circles) and the predominant grass, Yorkshire fog (*Holcus lanatus*) (open circles). Values shown are means (± standard error) where n = 10. The error bars show the least significant differences.

In kānuka, A_{max} values increased gradually from springtime and displayed a nearly 3-fold increase in autumn. This was followed by a rapid decline in A_{max} in winter. Thus, A_{max} varied to a greater extent in kānuka than in the grass. Interestingly, A_{max} values changed in summer and were found to be relatively low when the soil water content values were below 15 to 20 %. Seasonal changes in environment had a significant effect on relative stomatal limitation (L_{stom}), which was found to be relatively high in summer in both plant types (Figure 3.5E). In grass, the relative stomatal limitation ranged from 18.00 to 49.6 % whereas in kānuka, it ranged between 29.0 and 62.8 %.

Photosynthetic parameters, V_{cmax}, J_{max} and J_{max} / V_{cmax} ratio, were found to be highly dependent on temperature in both plant types. In grass, V_{cmax} declined at temperature above 28 °C and J_{max} decreased at temperatures above 25 °C. In kānuka, V_{cmax} and J_{max} declined at temperatures beyond 32 and 30 °C, respectively (Figure 3.6). Moreover, V_{cmax}, J_{max} and the J_{max} / V_{cmax} ratio were dependent on soil water content (θ) of both species.. Therefore, in order to resolve the combined effect of temperature and soil water content on photosynthesis for the grass and kānuka, a non-linear mixed effect model with both leaf temperature and soil water content as driving variables (e.g., Equation 2.5) was used. Results of the mixed effect models showed that the values of V_{cmax} and J_{max} were influenced significantly by leaf temperature and soil water content for the grass while leaf temperature was the only significant driving variable for kānuka (Table 3.1). The impact of soil water content was found to be more prominent on J_{max} than that on V_{cmax}.

The relationship between stomatal limitation and leaf temperature was linear and positive in both species. The relationship between stomatal limitation and soil water content was linear for the grass and curvilinear for kānuka. The combined effect of temperature and soil water content on stomatal limitation was resolved using a linear mixed effect model (Figure 3.7).

Stomatal limitation (L_{stom}) correlated negatively with A_{max} in the grass but not in kānuka (Figure 3.8). Parameters for these relationships are shown in Table 3.2.

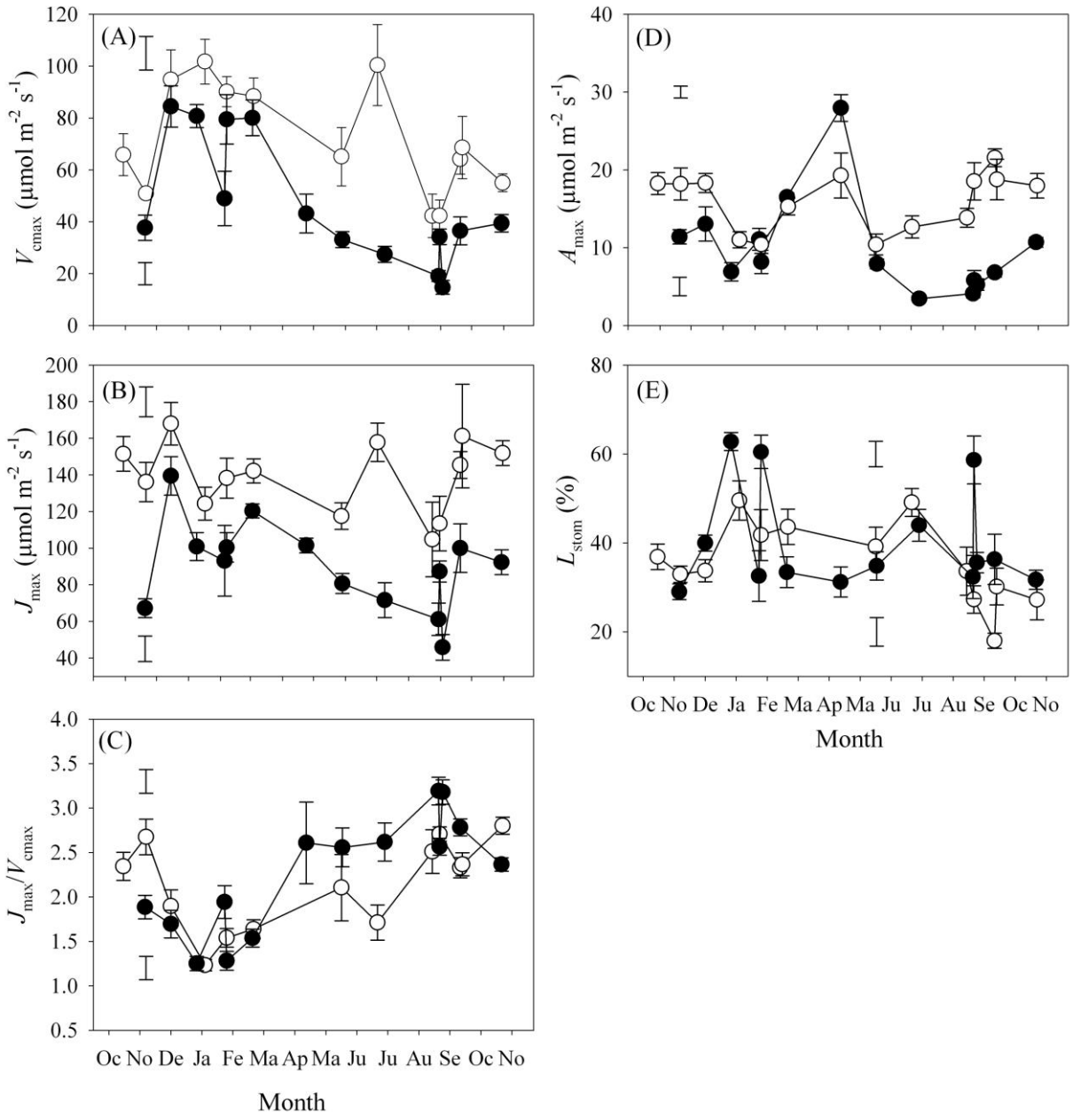

Figure 3.5 Seasonal variation in photosynthetic parameters calculated from A/C_i and A/Q responses in field grown kānuka (closed circles) and the grass (open circles) (A) V_{cmax}: maximum rate of ribulose-1, 5-bisphosphate (RuBP) carboxylation, (B) J_{max}: maximum rate of RuBP regeneration, (C) J_{max} /V_{cmax} ratio, (D) A_{max}: net CO_2 assimilation measured at saturating irradiance and ambient CO_2, (E) L_{stom}: limitation imposed by the stomata on the rate of photosynthesis. Values represent means (± standard error) of 6-10 estimates for each parameter and the error bars show the least significant difference.

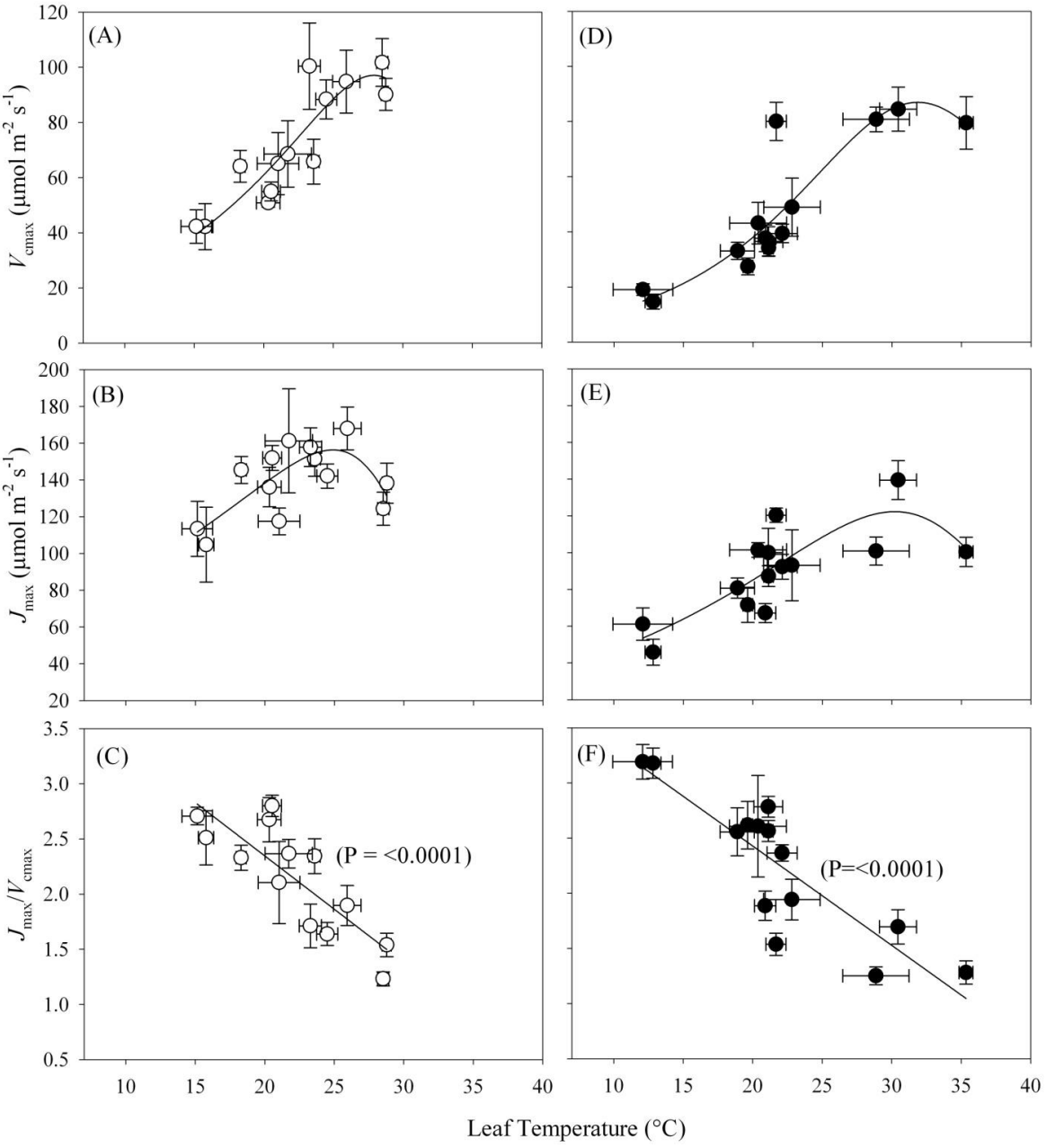

Figure 3.6 The relationship between seasonal variation in leaf temperature and the photosynthetic model parameters V_{cmax} (A), (D) and J_{max} (B), (E) displayed for the grass (open circles) and kānuka (closed circles), respectively. The lines are fitted to the data using the temperature response function of Leuning (2002) to estimate the parameters describing the temperature dependence of V_{cmax} and J_{max} at different temperatures. To resolve the combined effect of temperature and soil water content a non-linear mixed effect model was used - parameters describing the temperature response of V_{cmax} and J_{max} are shown in Table 3.1. Relationship between leaf temperature and the ratio of J_{max}/V_{cmax} for the grass and kānuka is shown in (C) and (F), respectively. A linear mixed effect model was also used for resolving the combined effect of temperature and soil water content on J_{max}/V_{cmax} ratio and the parameters describing the combined effects are shown in Table 3.2.

Table 3.1 Parameters obtained by fitting photosynthetic data using the repeated measures mixed effect models for V_{cmax} and J_{max} using leaf temperature (K) and soil water content as driving variables.

Species	Variables	$V_{cmax, 20}$ (µmol m^{-2} s^{-1})	H_{av} (kJ mol^{-1})	θ_c (%)	P value
Grass (*H. lanatus*)	Leaf temperature, Soil water content	60.09	56.5	17.3	0.04561
Kānuka (*K. ericoides*)	Leaf temperature	39.02	43.8		< 0.001
Species	**Variables**	$J_{max, 20}$ (µmol m^{-2} s^{-1})	H_{aj} (kJ mol^{-1})	θ_c (%)	**P value**
Grass (*H. lanatus*)	Leaf temperature, Soil water content	137.12	21.0	19.00	0.0003
Kānuka (*K. ericoides*)	Leaf temperature	84.30	21.3		< 0.001

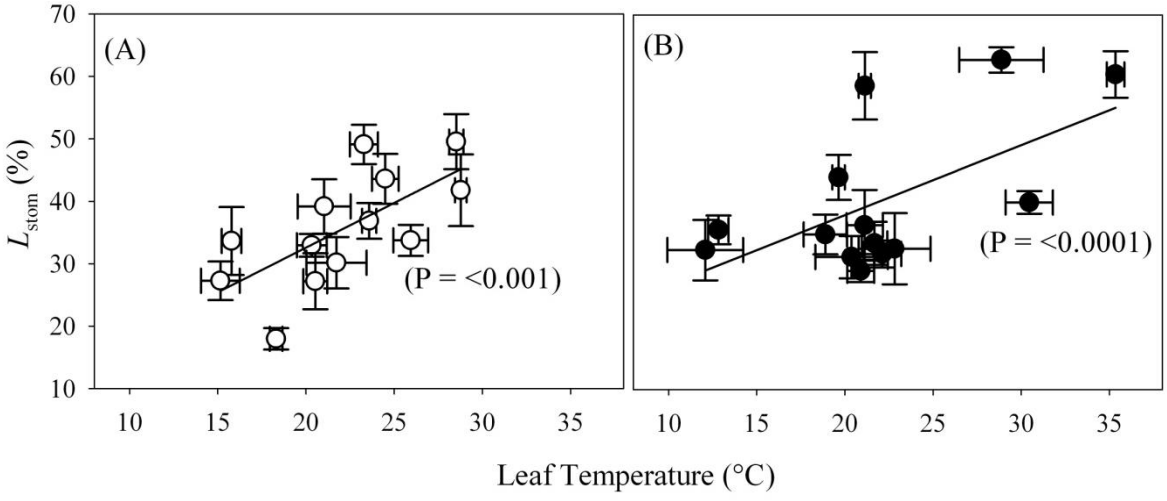

Figure 3.7 The relationship between seasonal variation in relative stomatal limitation, L_{stom} (%) and leaf temperature (°C) in the grass (A) and kānuka (B) described by a line of best fit using linear regression. To resolve the combined effects of temperature and soil water content, a linear mixed effect model was used - parameters from these regressions are shown in Table 3.2. Values represent the mean (± standard error) where n = 6-10.

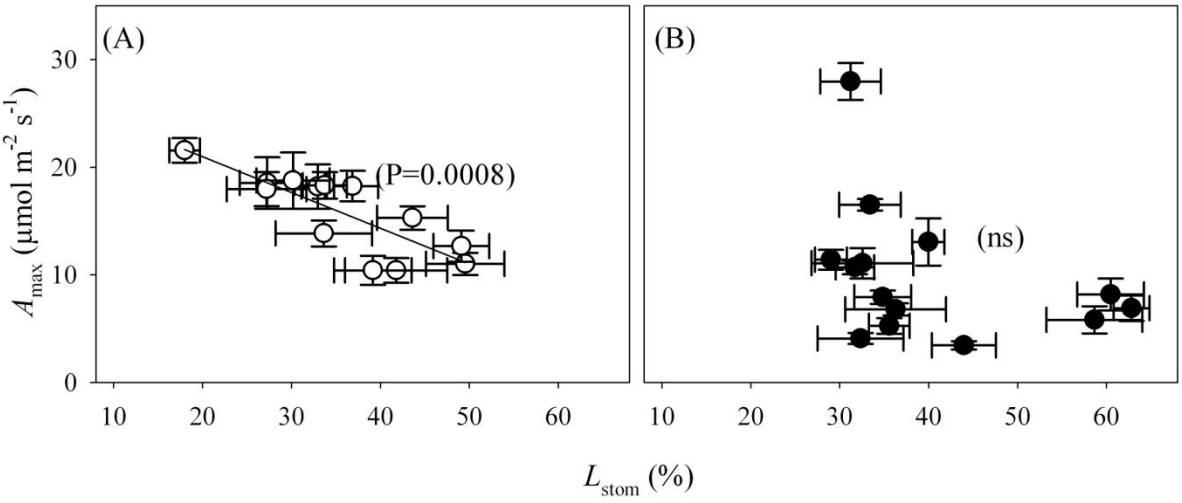

Figure 3.8 The influence of seasonal variation in stomatal limitation on seasonal changes in A_{max} for the grass (A) and kānuka (B). The significant linear regression parameter fitting this relationship is described in Table 3.2.

Figure 3.9 shows linear regression analyses between foliar characteristics and photosynthetic parameters. Photosynthetic parameters were dependent on specific leaf area and the sugar concentration. Astrong negative linear relationship between the sugar content and V_{cmax} and a strong negative linear relationship between the sugar content and J_{max} / V_{cmax} ratio were also observed in these species (for parameters see Table 3.3). The relationships between A_{max} and S and A_{max} and sugar content were different for the grass and kānuka. The relationship between A_{max} and S was negative in the grass whereas this relationship was positive in kānuka. Furthermore, A_{max} and sugar content correlated positively in the grass while a negative relationship was observed between A_{max} and sugar content in kānuka.

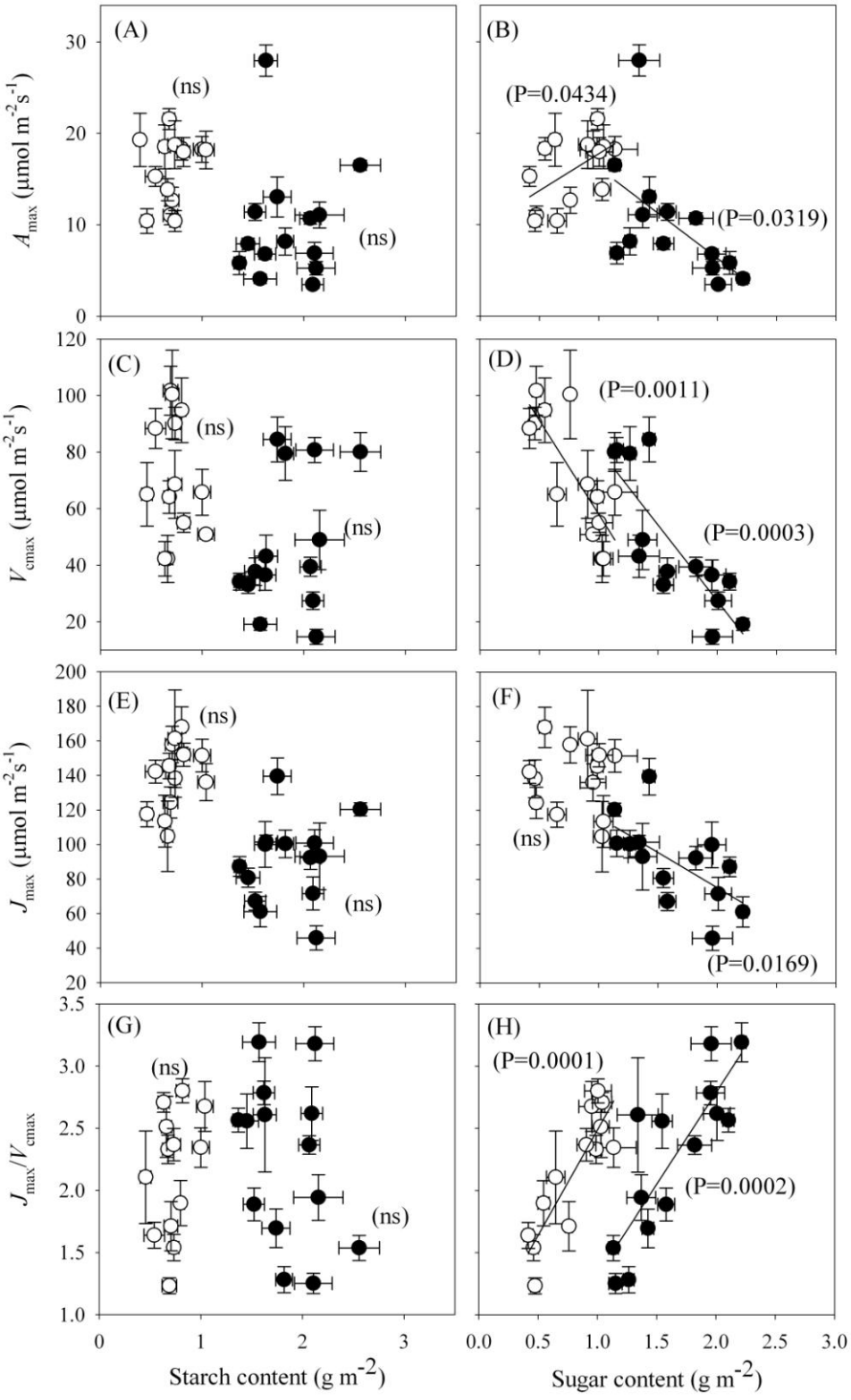

Figure 3.9 The relationship between A_{max} (A, B) V_{cmax} (C, D), J_{max} (E, F) and J_{max}/V_{cmax} ratio (G, H,) and starch and sugar contents in leaves of kānuka (closed circles) and the grass (open circles) sampled over a 12 month period. Each point is a mean value for each sampling date (n = 6-10). The parameters for the significant regressions are shown in Table 3.3.

Table 3.2 Significant linear regression parameters from relationships described in Figures 3.6, 3.7 and 3.8.

Figure	Regression relationship	r^2
	Grass	
3.6C	J_{max}/V_{cmax} = (-0.170 x -2.740) T_l (°C) + 5.990	0.52
3.7A	L_{stom} (%) = 1.57 T_l (°C) + 0.243	0.30
3.8A	A_{max} (µmol m^{-2} s^{-1}) = -0.332 L_{stom} (%) + 27.610	0.65
Figure	**Regression relationship**	**r^2**
	Kānuka	
3.6F	J_{max}/V_{cmax} = (-0.070 x 1.042) T_l (°C) + 3.329	0.60
3.7B	L_{stom} (%) = 2.029 x 42.256 T_l (°C) - 10.12	0.32

Table 3.3 Significant linear regression parameters from relationships described in Figures 3.9.

Figure	Regression relationship	r^2
	Grass	
3.9 B	A_{max} (µmol m^{-2} s^{-1}) = 8.067 sugar (g m^{-2}) + 9.689	0.30
3.9 D	V_{cmax} (µmol m^{-2} s^{-1}) = -66.42 sugar (g m^{-2}) + 124.50	0.64
3.9 H	J_{max}/V_{cmax} = 1.69 sugar (g m^{-2}) + 0.79	0.76
Figure	**Regression relationship**	**r^2**
	Kānuka	
3.9 B	A_{max} (µmol m^{-2} s^{-1}) = -9.86 sugar (g m^{-2}) + 26.050	0.33
3.9 D	V_{cmax} (µmol m^{-2} s^{-1}) = -53.73 sugar (g m^{-2}) + 134.89	0.68
3.9 F	J_{max} (µmol m^{-2} s^{-1}) = -40.86 sugar (g m^{-2}) + 156.91	0.39
3.9 H	J_{max}/V_{cmax} = 1.47 sugar (g m^{-2}) - 0.16	0.70

3.3.3 Respiration

Values of R_{10} and E_o for the grass and kānuka were calculated using Equation 2.4, from the short-term temperature response curves measured throughout the year. R_{10} and E_o values changed significantly ($P < 0.001$) with season and the trend in their seasonal response was found to be similar in both plant types. R_{10} increased throughout the autumn, winter and early spring and then declined from mid-spring through to late summer. E_o increased from mid-summer to mid-winter and declined from late winter to early summer. Therefore, the relationship between R_{10} and E_o was correlated inversely in both plant types. R_{10} values ranged from 0.16 to 0.47 µmol m^{-2} s^{-1} and 0.38 to 1.06 µmol m^{-2} s^{-1} for the grass and kānuka respectively (Figure 3.10A). R_{10} values in the grass were found to be the highest during late winter and early spring (August 2008 to October 2008) whereas for kānuka they were higher in spring (October 2008). E_o ranged from 43.7 to 79.7 kJ mol^{-1} in the grass and 47.0 to 88.9 kJ mol^{-1} in kānuka (Figure 3.10 B). Relatively high values of E_o were observed from late summer to mid-winter and low values from late winter to mid-summer for both the plant types. Plant respiration in response to the seasonal changes in growth temperatures (R_{growth}) varied significantly ($P < 0.001$) in both the plant types (Figure 3.10C). R_{growth} values increased from early-spring to early summer and then decined in mid summer (January-February) and early winter (May-July) for both plant types. Seasonal variability in respiration was described well by the model with leaf temperature and soil water content (θ) as driving variables (Equation 2.6), displaying a significant influence of these driving variables on respiratory parameters (Table 3.4). The sensitivity of respiratory parameters to seasonal changes in environmental variables was found to differ between plant types. Analysis of non-linear mixed effect model revealed a higher sensitivity of respiratory parameters to seasonal changes in soil water content in the grass than that of kānuka. R_{10} values were positively correlated with soluble sugar content in both species, while no significant relationship was

observed between starch content and R_{10}. A negative relationship between starch and E_o values was observed in the grass but not in kānuka.

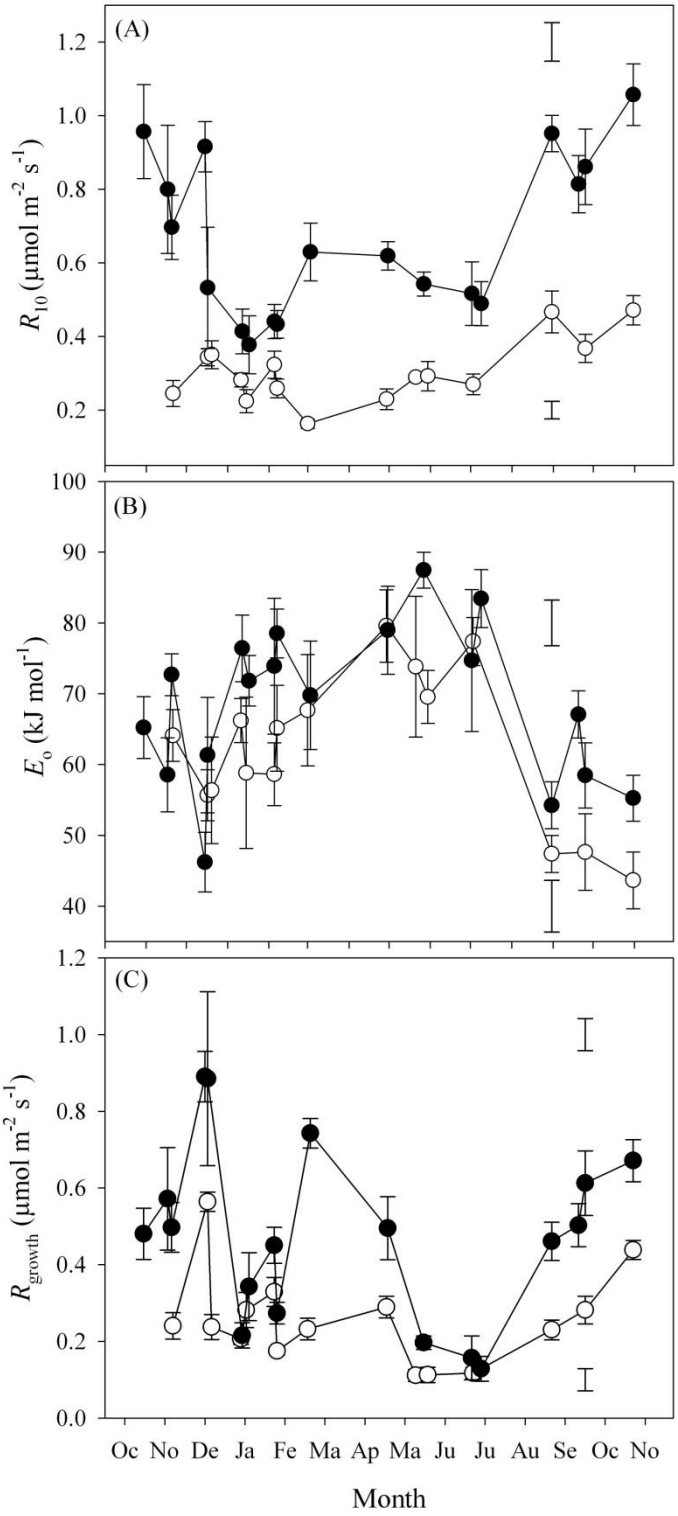

Figure 3.10 Seasonal variation in temperature response parameters of respiration R_{10} (A) E_o (B) and R_{growth} (C) in field grown kānuka (closed circles) and the grass (open circles) over the course of a 12 months period measured over the range of 8-25 °C. All values are means ± standard error where, n = 6 –10 and the error bars show the least significant difference.

Table 3.4 Parameters obtained by fitting respiration response data using the repeated measures non-linear mixed effect model described by Equation 2.6, using leaf temperature (K) and soil water content as variables for kānuka and the grass.

Species	Variables	R_{10} (μmol m^{-2} s^{-1})	E_o (kJ mol^{-1})	θ_c (%)	P value
Grass (*H. lanatus*	Leaf temperature, Soil water content	0.51	66.3	50.00	< 0.001
Kānuka (*K. ericoides*)	Leaf temperature, Soil water content	0.76	57.4	26.00	< 0.001

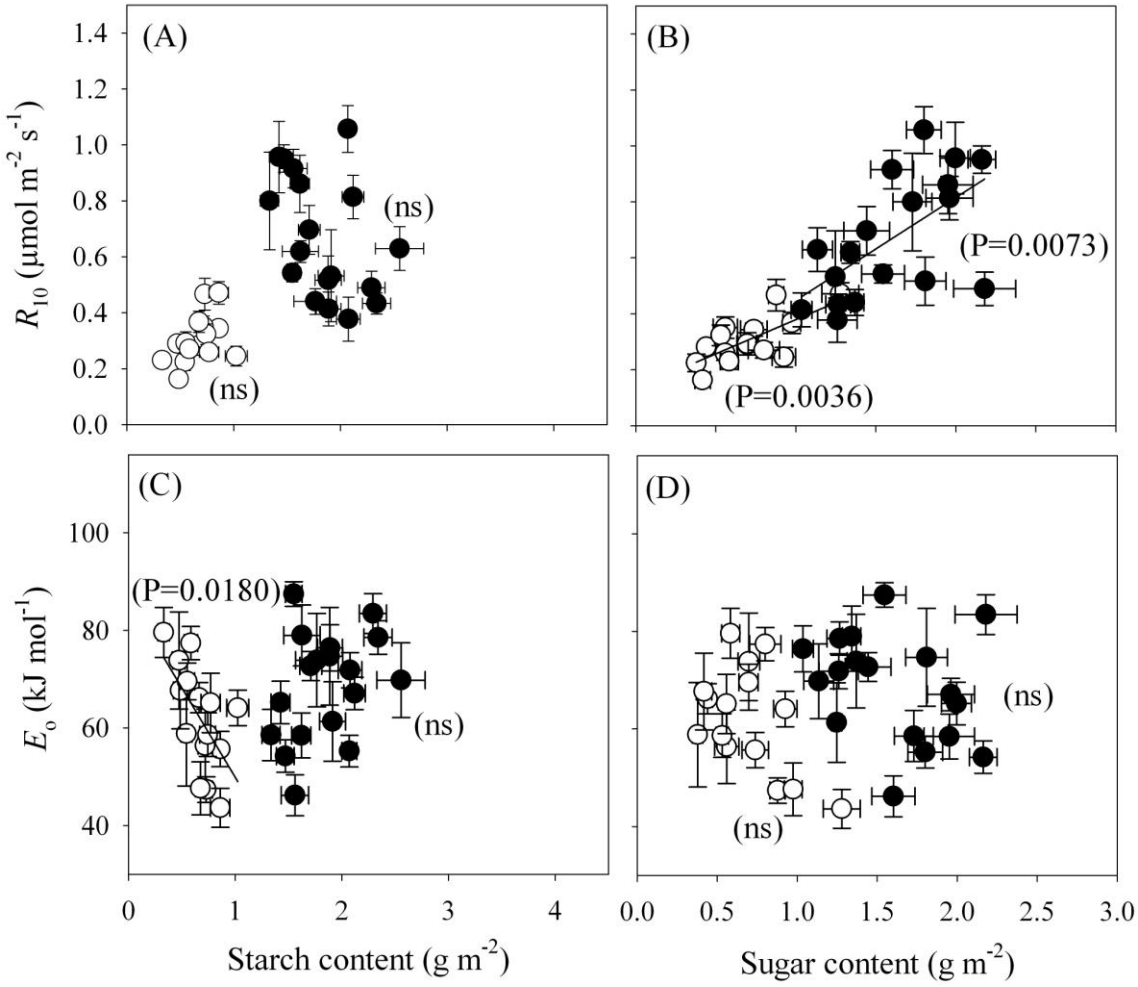

Figure 3.11 The relationship between R_{10} (A, B) and E_o (C, D) and starch and sugar content in leaves of kānuka (closed circles) and the grass (open circles) sampled over a 12 month period. Each point is a mean value for each sampling date (n = 6-10). The significant regression parameters fitting this relationship are shown in Table 3.5.

Table 3.5 Significant linear regression parameters from relationships described in Figure 3.11.

Figure	Regression relationship	r^2
	Grass	
3.11 B	R_{10} (μmol m^{-2} s^{-1}) = 0.245 sugar (g m^{-2}) + 0.135	0.49
3.11 C	E_o (kJ mol^{-1}) = -36.617 starch (g m^{-2}) + 86.559	0.36
Figure	**Regression relationship**	**r^2**
	Kānuka	
3.11 B	R_{10} (μmol m^{-2} s^{-1}) = 0.368 sugar (g m^{-2}) + 0.081	0.37

3.3.4 Acclimation of respiration to temperature

The seasonal (acclimated) response of respiration to ambient growth temperature (R_{growth}) showed a positive relationship with the minimum temperature of the previous day for both the plant types, which could be fitted using the Arrhenius function in Equation 2.4 (Figure 3.12). In Figure 3.12 this seasonal response is compared to the instantaneous thermal response of respiration in mid-winter and mid-summer. Differences between the mid-winter and mid-summer instantaneous temperature responses curves were relatively large at higher measuring temperatures. Importantly, the seasonal (acclimated) response sat between the mid-winter and mid-summer instantaneous temperature responses curves. Moreover, R_{10} values obtained from the seasonal response curve were also between the R_{10} values of the mid-winter and mid-summer instantaneous temperature responses curves for both plant types (Table 3.6).

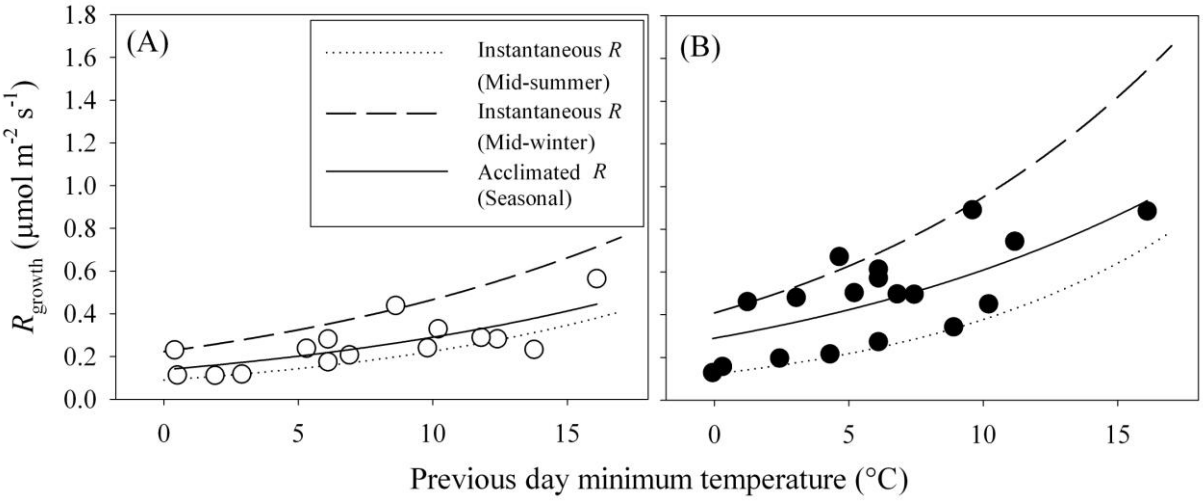

Figure 3.12 Relationships between area-based respiration (R_{growth}) and minimum temperature of the day preceding the measuring day in the grass (open circles) and kānuka (closed circles) sampled over different seasons. Apparent seasonal acclimation in the grass ($r^2 = 0.53$, P = 0.002) and kānuka ($r^2 = 0.50$, P = 0.001) is demonstrated by fitting Equation 2.6 (solid line). The dashed lines show the instantaneous response of respiration to temperature in mid-summer (January 2007) and mid-winter (August 2007) and this displays the extent of seasonal thermal acclimation in both plant species. The significant parameters fitting these relationships are shown in Table 3.6.

Table 3.6 Values of respiratory temperature response parameters in the grass and kānuka displayed in Figure 3.12.

	Grass		**Kānuka**	
Temporal variations	R_{10} (µmol m^{-2} s^{-1})	E_o (kJ mol^{-1})	R_{10} (µmol m^{-2} s^{-1})	E_o (kJ mol^{-1})
Mid-Winter	0.47	47.4	0.95	54.2
Mid-Summer	0.23	58.8	0.38	71.8
Seasonally acclimated	0.29	47.6	0.61	47.6

In order to examine the apparent timing of acclimation to changes in environmental variables, previous day minimum air temperature and soil water content measurements were averaged across a varying number of preceding days (from 1-day to 300-days) and these averaged values were used to examine relationships with respiratory parameters (Figure 3.13). Regression analysis confirmed a strong relationship exists between the respiratory parameters

and previous minimum air temperature and soil water content across a varying number of proceeding days. A strong negative relationship was observed between previous minimum temperatures and R_{10}, while a strong positive relationship was observed with E_o values for both plant types (Figure 3.14). Even though the relationship between R_{10} and previous minimum temperatures was found to be significant ($P< 0.05$) over a range of averaging windows from 40 days to 150 days, the strongest relationship was observed with a preceding 125-day average in both the grass and kānuka (Figure 3.14A and 3.14B). Similarly for E_o, the relationship with previous minimum temperature was significant for the previous 100 to 250 day average and the strongest relationship was observed with a 150-day average in both the plant types (Figure 3.14C and 3.14D).

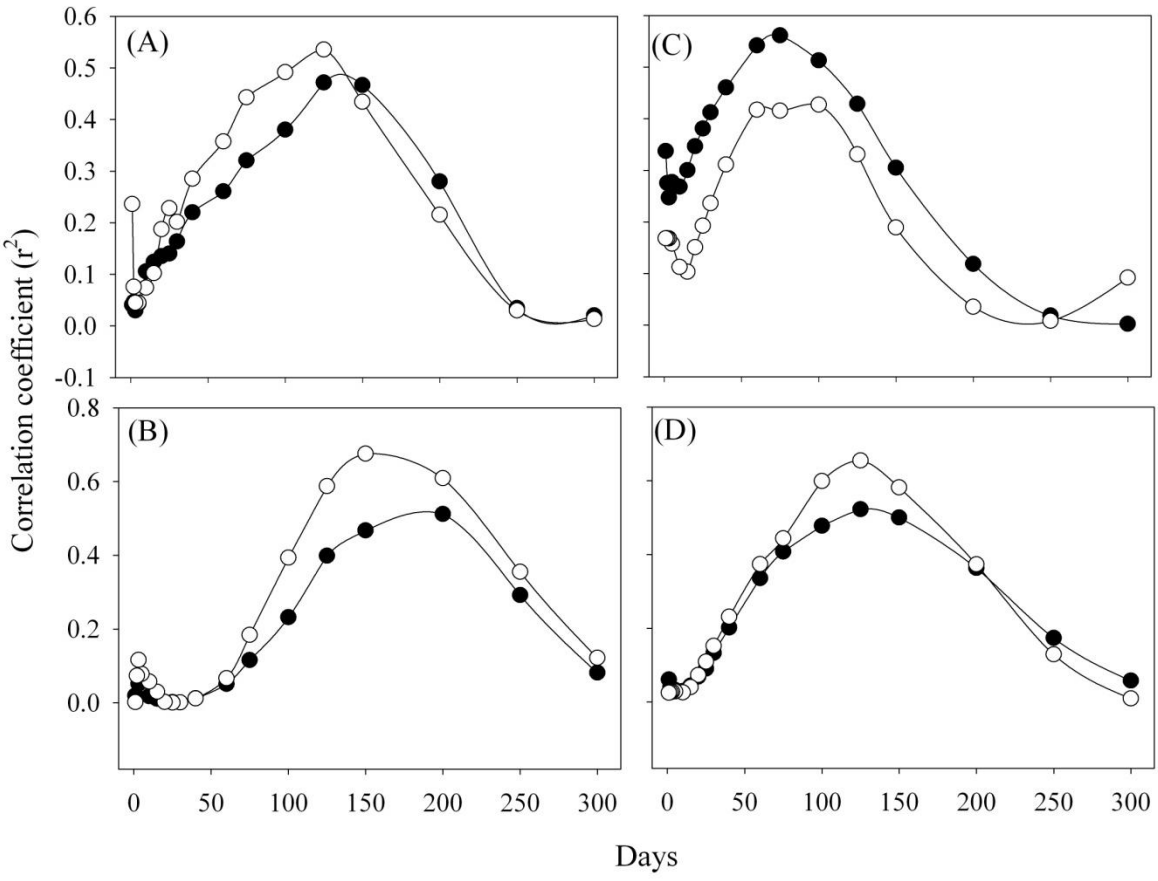

Figure 3.13 Correlation coefficients of the relationship between minimum temperature (T_{min}) and R_{10} (A) and E_o (B) and volumetric soil water content (θ) and R_{10} (C) and E_o (D) for the field grown grass (open circles) and kānuka (closed circles). Values are plotted as a function of the time window (days) used to calculate the effect of preceding average night temperature and soil water content experienced by the plants.

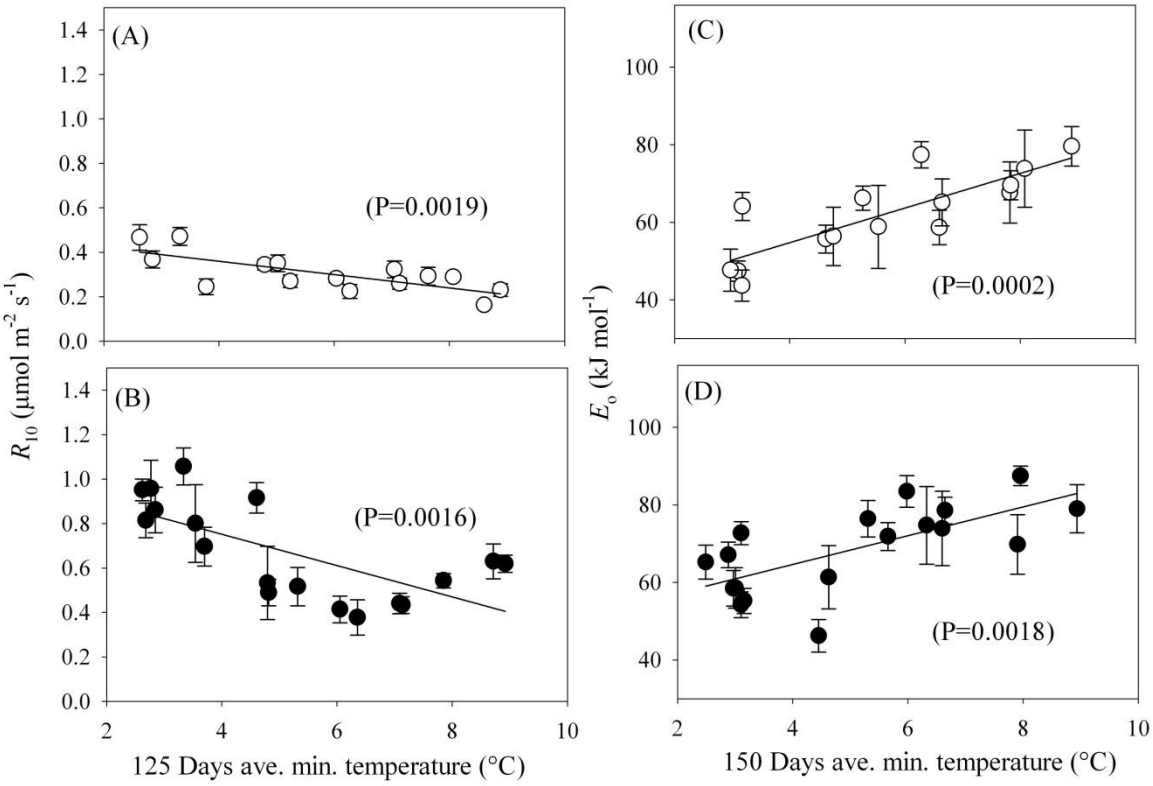

Figure 3.14 Linear relationships between R_{10} (A, B) and E_o (C, D) with previous 125-day and 150-day minimum temperature average (T_{min}) in the grass (open circles) and kānuka (closed circles) sampled over different seasons. The linear regression parameters fitting this relationship are shown in Table 3.7

Regression analysis found no significant relationship between the actual soil water content at the time of sampling and R_{growth} in either plant type. R_{10} showed a strong positive relationship with the actual soil water content in both plant types (Figure 3.15A and 3.15B) while E_o did not (Figure 3.15C and 3.15D). There was a strong correlation between both R_{10} and E_o and previous soil water content in both plant types (Figure 3.16C & Figure 3.16D). Interestingly, the relationship between R_{10} and previous soil water content was found to be positive and the strongest relationship was observed with a preceding 60-day average, while E_o values showed a negative relationship and the strongest correlation was observed with the average volumetric soil water content over the preceding 125 days (Figure 3.16).

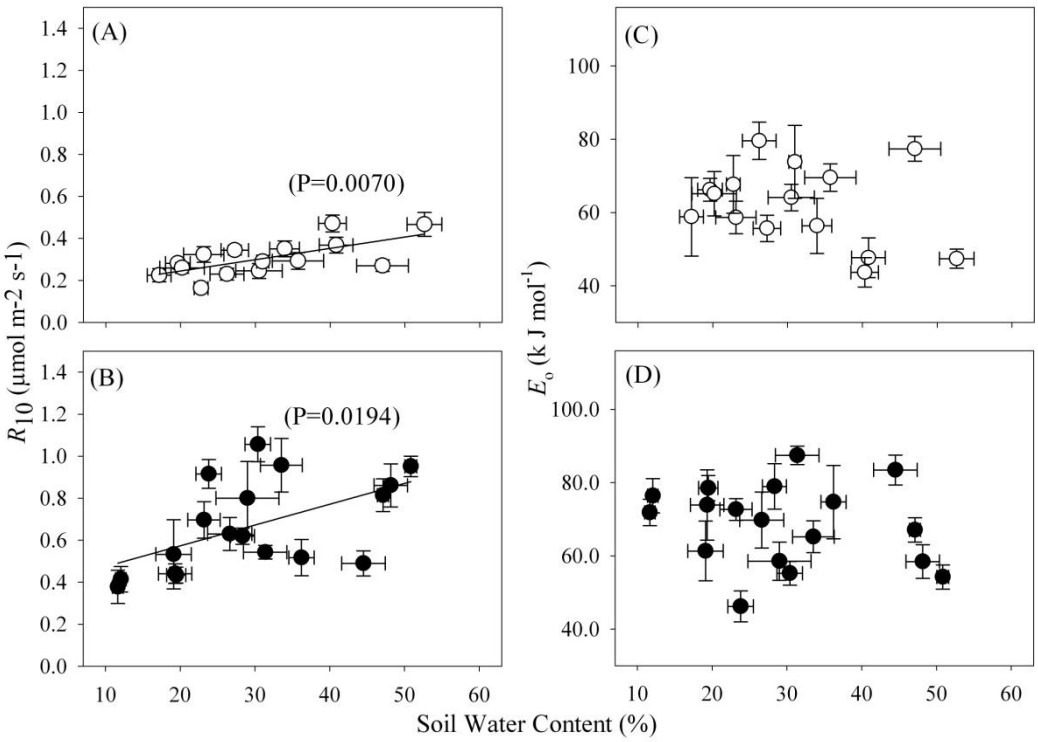

Figure 3.15 The relationship between soil water content (θ) (on the day of sampling) and R_{10} (A and B) and E_o (C and D) in the grass (open circles) and kānuka (closed circles) sampled over a 12-month period. Each data point represents the mean of each sampling date. The linear regression parameters fitting the significant relationships are shown in Table 3.7

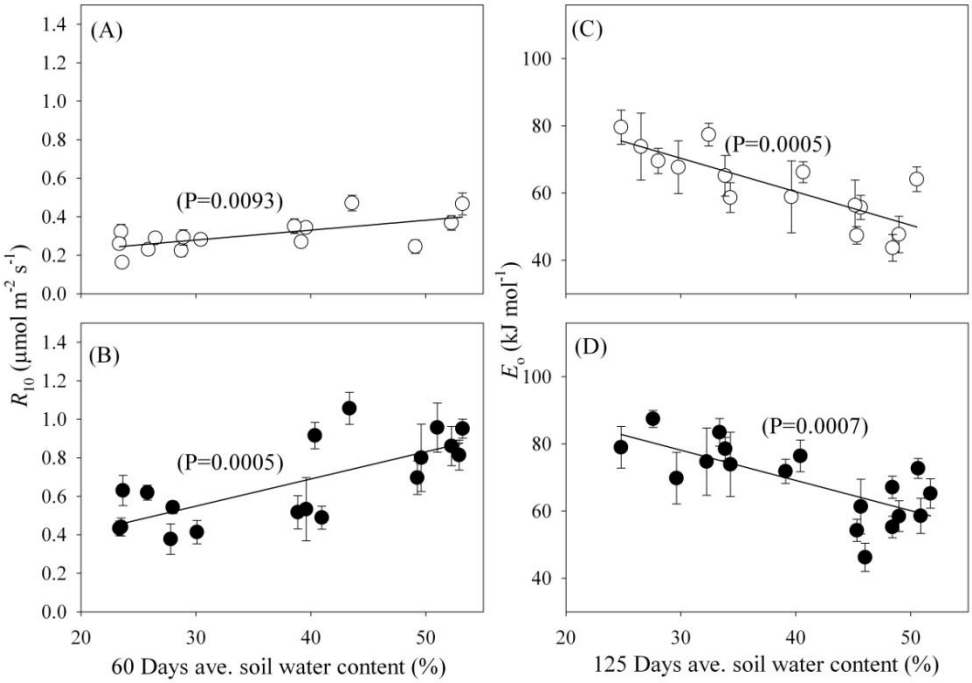

Figure 3.16 Linear relationships between the previous days soil water content (θ) and R_{10} (A, B) and E_o (C, D) for the field grown grass (open circles) and kānuka (closed circles) sampled over different seasons. The linear regression parameters fitting this relationship are shown in Table 3.7

Table 3.7 Significant linear regression parameters from relationships described in Figure 3.12, Figure 3.13, Figure 3.15 and Figure 3.16.

Figure	Regression relationship	r^2
	Grass	
3.13A	R_{10} (µmol m^{-2} s^{-1}) = -0.0300 previous 125 day T_{min} (°C) + 0.4797	0.54
3.13C	E_o (kJ mol^{-1}) = 4.4651 previous 150 day T_{min} (°C) + 36.8764	0.68
3.15A	R_{10} (µmol m^{-2} s^{-1}) = 0.0007 θ (%) + 0.0095	0.36
3.16A	R_{10} (µmol m^{-2} s^{-1}) = 0.0051 previous 60 day θ (%) + 0.1247	0.42
3.16C	E_o (kJ mol^{-1}) = -0.9966 previous 125 day θ (%) + 100.2525	0.66
Figure	**Regression relationship**	**r^2**
	Kānuka	
3.13B	R_{10} (µmol m^{-2} s^{-1}) = -0.0705 previous 125 day T_{min} (°C) + 1.0336	0.47
3.13D	E_o (kJ mol^{-1}) = 3.7104 previous 150 day T_{min} (°C) + 49.7816	0.47
3.15B	R_{10} (µmol m^{-2} s^{-1}) = 0.0099 θ (%) + 0.3749	0.30
3.16B	R_{10} (µmol m^{-2} s^{-1}) = 0.0141 previous 60 day θ (%) + 0.1250	0.54
3.16D	E_o (kJ mol^{-1}) = -0.9005 previous 125 day θ (%) + 105.1439	0.52

3.3.5 Changes in the R_{growth}/A_{max} ratio

The net effect of seasonal changes in A and R is shown in the response of the R_{growth}/A_{max} ratio. This was found to be highly variable in kānuka, showing a decline from summer to autumn (December 2007 to May 2008) followed by a rapid increase in winter and then remaining high until late spring. The R_{growth} /A_{max} ratio of the grass was appreciably lower than for kānuka, and remained relatively stable from late summer to mid-spring (February 2008 to September 2008) after which it increased in spring and summer (Figure 3.17).

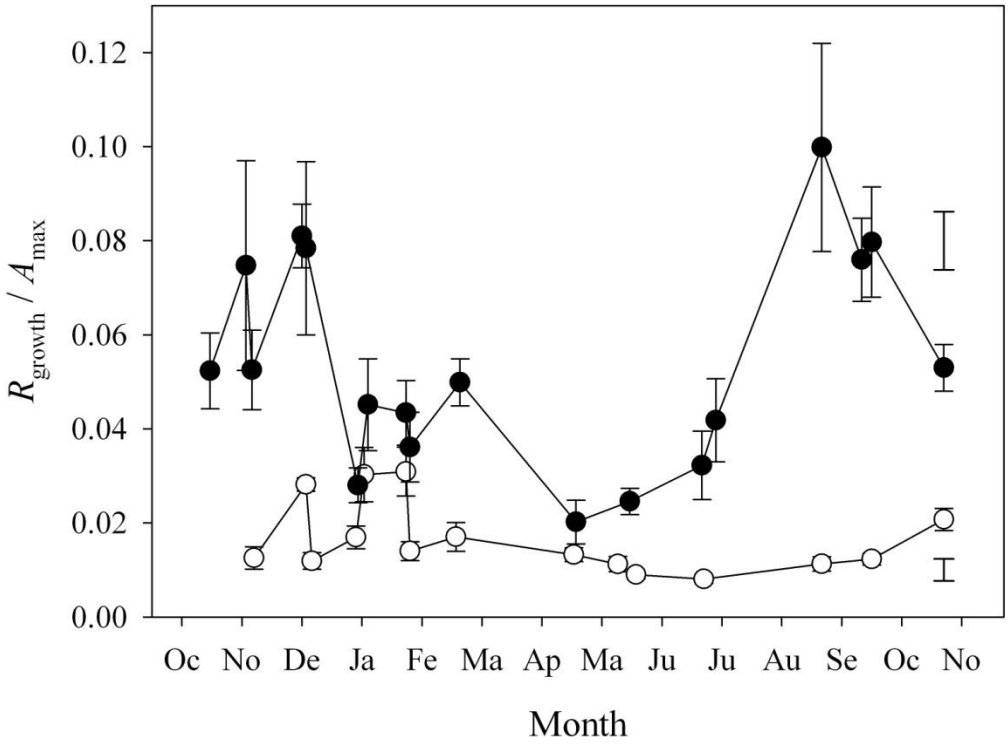

Figure 3.17 Seasonal variation in the ratio of dark respiration at the growth temperature (R_{growth}) to light saturated photosynthesis at ambient CO_2 (A_{max}) for the field grown grass (open circles) and kānuka (closed circles) measured over the course of a 12 months period. n = 6-10. All values are means ± standard error.

3.4 DISCUSSION

Seasonal environmental variation at the site influenced the gas exchange characteristics of the grass and kānuka. The seasonal patterns of gas exchange were found to be generally similar for both the plant types, although the extent of their response to growth temperature and soil water content differed.

3.4.1 Response of photosynthesis to temperature

Growth temperature can affect photosynthesis directly and/or indirectly (Lloyd and Farquhar 2008). Direct temperature effects on photosynthesis involving changes in V_{cmax} and J_{max} were clearly manifested in both the plant types. An indirect effect of temperature in reducing photosynthesis due to a reduction in stomatal conductance was also found. Even though stomatal limitation in both the plant types showed a significant positive relationship with

seasonal changes in growth temperature, the impact of stomatal limitation on photosynthesis was found to be significant only in the grass (Figure 3.8). . Therefore, it is important to identify that the direct effect of temperature regulating biochemical changes is very sensitive to changes in intercellular CO_2 concentration exerted by stomatal limitations at elevated temperature, and the combined effect of this stomatal (indirect) and non-stomatal (direct) effect represents the actual response of photosynthesis to higher temperature.

Seasonal variation in growth temperature resulted in a nearly six-fold increase in V_{cmax} and a three-fold increase in J_{max} for kānuka. V_{cmax} and J_{max} values increased nearly 2.5 fold and 1.5 fold in the grass. Relatively high values of V_{cmax} were more consistently observed during warmer months i.e., from December to March in the grass and kānuka. Seasonal variation in photosynthetic parameters is strongly dependent on leaf temperature and this response is explained by changes in the maximum air temperature experienced by these plants on the day of the measurement over the period of this experiment. This finding is consistent with previous temperature response studies (Medlyn *et al.* 2002a; Xu and Baldocchi 2003; Ow *et al.* 2010; Way and Oren 2010) showing a strong positive correlation of V_{cmax} and J_{max} with increasing leaf temperatures.

The findings of this field study also show that the ratio of the capacities of ribulose-1,5-bisphosphate (RuBP) regeneration to RuBP carboxylation (J_{max} / V_{cmax}) is seasonally variable, with relatively high values during colder months and low values in warmer months in the grass and kānuka (Figure 3.5C). This finding is also consistent with several previous studies (Hikosaka *et al.* 1999; Onoda *et al.* 2005a; Onoda *et al.* 2005b; Hikosaka *et al.* 2006; Ow *et al.* 2010). However, other findings do not show seasonal changes in the J_{max} / V_{cmax} ratio in mature maritime pine (*Pinus pinaster*, Medlyn *et al.* (2002b)) and in evergreen oak (*Quercus lobata* and *Quercus agrifolia*, Hollinger (1992)). The imbalance in the ratio of RuBP regeneration to RuBP carboxylation is due to differences in the temperature sensitivity of these processes because J_{max}

has a lower optimum temperature than V_{cmax} (Dreyer *et al.* 2001; Urban *et al.* 2007). In addition, these results provide confirmation of previous findings for kānuka and a grass that was observed in the previous chapter.

It is noteworthy that seasonal variation in J_{max} was relatively narrow when compared to the wide range of variation observed in V_{cmax}. At low temperatures, V_{cmax} declined far more than did J_{max} and hence the seasonal variation in J_{max} / V_{cmax} ratio was higher in colder months compared to warmer months. This reveals the limitation of RuBP regeneration on photosynthetic rate (Hikosaka 1997; Hikosaka *et al.* 1999; Onoda *et al.* 2005a). This seasonal variation in biochemical response is likely associated with the changes in the allocation of photosynthetic proteins (Onoda *et al.* 2005b; Yamori *et al.* 2005) and/or differences in partitioning of nitrogen between these two processes, because nitrogen content changes seasonally (Medlyn *et al.* 2002b) and photosynthetic capacity is closely associated with nitrogen content (Evans 1989; Hikosaka and Hirose 2000).

Variation in activation energies has been reported among species and along climatic gradients (Medlyn *et al.* 2002a; Kattge and Knorr 2007; Dillaway and Kruger 2010). In this study it was also observed that the activation energy of V_{cmax} is much higher than that of the J_{max} in both the plant types. This finding concurs with those of Onoda *et al.* (2005a) and Kositsup *et al.* (2009) who identified that declining J_{max} / V_{cmax} ratio with increasing temperature is due to greater activation energy for V_{cmax} than for J_{max}. Photosynthesis can function effectively between 7 to 40 °C in most plant species (Campbell *et al.* 2007; Sage and Kubien 2007) and an optimum temperature ranging from 15 to 30 °C is common among C_3 plants (Larcher 2003). In the present study, the thermal optimum of photosynthesis for both the species was within this range (i.e., 18 °C for the grass and 23 °C for kānuka). Changes in growth conditions may lead to changes in the thermal optimum that subsequently result in 'thermal acclimation'. The mechanisms of the response of thermal acclimation to cold temperatures include enhanced electron transport

capacity, enhanced Rubisco content, and enhanced sugar and starch synthesis, while acclimation to high temperature include respiratory decline, increased electron transport capacity and synthesis of the heat stable enzyme Rubisco activase (Sage and Kubien 2007). Recent studies showed limited photosynthetic acclimation to increasing temperature for species from cold climate zones (Atkin *et al.* 2006b; Ow *et al.* 2008a; Dillaway and Kruger 2010; Ow *et al.* 2010). In this study it appears that the grass and kānuka display low thermal acclimation potential to increasing temperature. This finding is comparable with previous findings showing that thermal acclimation of photosynthesis is more limited for grasses, shrubs and trees grown in cold climates compared to their acclimation potential in respiration.

3.4.2 Response of photosynthesis to soil water content

Seasonal variation in soil water content can influence photosynthesis via indirect (stomatal) and direct metabolic (RuBP and ATP suppy) regulation (Escalona *et al.* 1999; Tezara *et al.* 1999; Medrano *et al.* 2002; Lawlor 2002b). V_{cmax} displayed a significant negative relationship with seasonal increases in soil water content while J_{max} remained relatively constant in the grass and kānuka. This result indicates that the sensitivity of these photosynthetic parameters to varying soil water content is distinctly different. These results are likely associated with (i) changes in the concentration and or activity of enzymes involved in carboxylation such as Rubisco (Flexas *et al.* 2004; Lawlor and Tezara 2009), (ii) the maintenance of RuBP regeneration capacity through supply of ATP or NADPH, or (iii) retaining the enzymatic activity of photosynthetic carbon reduction cycle enzymes such as sedoheptulose-1,7- bisphosphatase and fructose-1,6-bisphosphatase (Parry *et al.* 2002; Lawlor 2002b; Flexas *et al.* 2004). Moreover, this result corroborates previous findings indicating that Rubisco does not limit photosynthesis until metabolic limitation to photosynthesis occurs due to severe or long-term water deficit (Flexas *et al.* 2006; Hu *et al.* 2009).

The marked correlation of soil water content on V_{cmax} and the lack of correlation on J_{max} resulted in a significant increase in the J_{max} / V_{cmax} ratio with increasing soil water content, indicating that the optimum soil water content for J_{max} is higher than that of V_{cmax}. Previous studies recognised that RuBP carboxylation and regeneration need a substantial amount of nitrogen to maintain high photosynthetic capacity (Hikosaka 1997) and reallocation of nitrogen from non-limiting to limiting processes occurs in plants to use nitrogen efficiently (Hikosaka and Terashima 1995). Therefore, the change in J_{max} / V_{cmax} with respect to soil water content is also likely linked to differences in partitioning of nitrogen between these two processes and/or changes in the allocation of photosynthetic proteins (Onoda *et al.* 2005b; Yamori *et al.* 2005).

Chaves (1991) reported that external factors such as water availability could influence the size of the carbohydrate pool, as water deficit induces an accumulation in sugar and a decrease in starch content in leaves. Results of the present experiment showed a slight decline in the starch content only in the leaves of the grass, but a concurrent decrease in sugar was not observed during warmer months. Kānuka displayed an increase in starch and a decrease in sugar content when soil water content was low. The temperature –mediated increase in photosynthetic capacity is likely associated with lower concentration of carbohydrate during warmer months and this finding is consistent with previous findings (Azcón-Bieto and Osmond 1983; Turnbull *et al.* 2002a). Moreover, the contrasting changes in carbohydrate contentare likely to be a species-specific plant response with respect to their relative sensitivity to changing environmental conditions and different developmental stages of growth.

3.4.3 Response of photosynthesis to temperature and soil water content

The non-linear mixed effect models were effective to resolve the entangled effect of temperature and soil water content on photosynthetic parameters. The response of photosynthesis to temperature and soil water content in the field was well described by the model in equation 2.5.

When the long-term seasonal variability in photosynthesis was described by non-linear mixed effect models with leaf temperature and soil water content as driving variables, the values of $V_{cmax,\ 20}$ and $J_{max,\ 20}$ for the grass generated were relatively higher while the activation energies of V_{cmax} and J_{max} generated were slightly lower, than that of values observed in the controlled growth cabinet experiment (Chapter 2). Relatively high $V_{cmax,\ 20}$ and $J_{max,\ 20}$ values for the field-grown grass indicates that the photosynthesis in the grass is high under a wide range of seasonal conditions. For kānuka, $V_{cmax,\ 20}$ and $J_{max,\ 20}$ were within the range of values observed in the controlled experiment but the corresponding activation energies were much lower. However, the values of V_{cmax} and J_{max} estimated at 20 °C for kānuka were slightly lower than V_{cmax} = 47.3 ± 1.9 μmol m^{-2} s^{-1} and J_{max} = 94.2 ± 3.7 μmol m^{-2} s^{-1} previously observed for mānuka and kānuka in the field conditions (Whitehead *et al.* 2004b).

Interestingly, the ratio of $J_{max,\ 20}$ / $V_{cmax,\ 20}$ was found to be similar for both the species under field and controlled growth cabinet conditions (i.e., 2.28 and 2.46 for the grass and 2.23 and 2.16 for kānuka, respectively). In the same way, if activation energies are compared, the values of H_{av} and H_{aj} of the field-grown grass and kānuka were found to be closer to the values of H_{av} and H_{aj} observed under controlled growth cabinet experiment. When soil water content fell below the threshold parameter θ_c, this resulted in a decrease in V_{cmax} (at values of θ below 17.3 %) and J_{max} (at values of θ below 19 %) for the grass but not for kānuka. Values of θ_c were reached during summer (early January to late February) (Figure 3.3). However, under controlled conditions (Chapter 2), values of V_{cmax} and J_{max} at 20 °C declined when soil water content fell below 17 % and 18 %, respectively for the grass and 23.6 % for both V_{cmax} and J_{max} at 20 °C for kānuka. These findings suggest that V_{cmax} and J_{max} of both plant types are insensitive to soil water content until the soil is dry.

This study suggests that the influence of soil water content on the seasonal variation in photosynthetic parameters is strong and unavoidable, particularly in summer and further

indicates that there is a pressing need for considering the effect of soil water content on photosynthetic parameters when developing models. A significant reduction in A_{max} was observed only in the grass at very low soil water contents during summer, however A_{max} values were also found to be relatively low at very high soil water contents in kānuka. The grass displayed a linear increase in A_{max} when soil water content exceeded 48 %, while kānuka showed a curvilinear response with a peak A_{max} around 20 % soil water content. The response of A_{max} to soil water content shows that kānuka is much more tolerant of low soil water content than the grass. However, it is interesting to note that the optimum soil water content observed for the grass and kānuka under field condition was different from the optimum soil water content values observed under controlled conditions (Chapter 2).

3.4.4 Response of respiration to temperature

Respiration is strongly sensitive to short-term changes in temperature in the grass and kānuka, but they exhibited considerable seasonal acclimation. In this study, respiration increased with ambient temperature (i.e., the previous night's minimum field temperature) in both species, but the seasonal response was less in magnitude compared to the instantaneous temperature response (Figure 3.12). The difference in respiration observed in winter and summer is most likely due to the differences in the basal rate of respiration. This finding is comparable with the results of a recent study on the impact of summer drought on the temperature response of respiration in beech seedlings (Rodríguez-Calcerrada *et al.* 2010) and several other studies displaying partial acclimation (Larigauderie and Korner 1995; Loveys *et al.* 2003).

Previous studies investigating acclimation of respiration to temperature have recognised that acclimation is not only associated with a lower rate of respiration at high temperature but it also may be associated with lower E_o (often expressed as Q_{10}) (Stockfors and Linder 1998; Atkin *et al.* 2000b; Atkin and Tjoelker 2003; Lambers *et al.* 2008). In the present study a strong

negative relationship between E_o and R_{10} was observed (Figure 3.18) which is comparable with the findings of Xu and Griffin (2006), Xu *et al.* (2007) and Searle *et al.* (2010). E_o was greater in autumn and early winter than in spring and summer in both kānuka and the grass. This is comparable with the findings of previous seasonal studies (Atkin *et al.* 2000b; Armstrong *et al.* 2008; Ow *et al.* 2008b; Ow *et al.* 2010) showing an increased temperature sensitivity of leaf respiration with cold temperatures under both controlled environment and field conditions. However, a correlation between the previous night's minimum field temperature and E_o was not significant in either of the plant types. According to Atkin *et al.* (2005b), the impact of seasonal variation on plant tissues and their concomitant acclimation response may subsequently alter E_o, likely attributable to changes in substrate availability, enzyme capacity, demand for respiratory energy and stage of development. In the present study, a substrate dependent change in E_o was not clear even though an inverse relationship between starch and E_o was observed in the grass. However, previous studies suggest that changes in E_o are likely due to changes in plant development (Atkin *et al.* 2005a; Marra *et al.* 2009) and other environmental variables such as drought (Bryla *et al.* 1997; Turnbull *et al.* 2001).

Seasonal variation in specific leaf area and non-structural carbohydrates can help explain changes in the rate of respiration. A significant inverse relationship was observed between R_{10} (on an area basis) and *S* for kānuka, showing that the rate of respiration rate is higher in thicker leaves during colder months and lower in thinner leaves during warmer months. Therefore, the rate of respiration is more closely related to leaf mass than leaf area. Previous studies have also demonstrated an increase in respiration due to an increased mitochondrial density resulting from the suppression of cellular expansion in the leaves during colder months (Reich *et al.* 1998; Turnbull *et al.* 2001; Armstrong *et al.* 2006a). Additionally, the influence of sugar content, acting as substrates for glycolysis and mitochondrial respiration is clearly demonstrated in the present study through a significant correlation between sugar content and the rate of respiration.

This is consistent with previous findings (Azcón-Bieto and Osmond 1983; Griffin *et al.* 2001; Turnbull *et al.* 2003; Xu and Griffin 2006; Ow *et al.* 2010). Unlike sugar, starch content did not show a significant correlation with the rates of respiration in either plant type. It is likely that starch is converted to soluble sugars (the direct substrate for respiration) when respiration demands are high, although a negative correlation was identified between E_o and starch content only in the grass. This is consistent with previous findings in tree species *Quercus rubra* and *Quercus prinus* growing in sites with varying soil water content (Turnbull *et al.* 2001). It is clear that carbohydrate status is an important regulatory feature in respiratory acclimation.

3.4.5 Response of respiration to soil water content

Respiration also responded to seasonal changes in soil water content. . The results show that R_{growth} was not influenced by soil water content, but R_{10} increased significantly with increasing soil water content in the grass (P=0.007) and kānuka (P=0.02). This positive correlation is comparable with the results of the experiment under controlled condition (Chapter 2) and previous studies demonstrating the impact of water deficit on plants at both leaf (Collier and Cummins 1996; Flexas *et al.* 2005; Ribas-Carbo *et al.* 2005; Flexas *et al.* 2006; Galmés *et al.* 2007; Atkin and Macherel 2009; Vassileva *et al.* 2009; Vassileva *et al.* 2011) and ecosystem (Bowling *et al.* 2002) scales. At the onset of summer, a significant reduction in R_{10} values was observed and it remained relatively low throughout the season in both the plant types, indicating the influence of low soil water content on respiratory capacity. Soil water content recorded during this period ranged between 10 to 25 %. However, R_{10} values increased during late autumn and peaked in winter when soil water content was consistently above 35 %. This effect may be mediated through photosynthesis: lower rates of photosynthesis (Lawlor and Cornic 2002; Flexas *et al.* 2004) produce lower amounts of carbohydrates during drought, which starves

respiration. Rodríguez-Calcerrada (2010) demonstrated the impact of soil water content on beech seedlings experiencing summer drought.

The activation energy of respiration (E_o) was found to be relatively low during warmer months (from spring to summer) and high in cooler months (from autumn to early winter). This finding is comparable with the results of several seasonal studies on evergreen species under field conditions [*Chamaecyparis obtusa* (Paembonan *et al.* 1991); *Eucalyptus pauciflora* (Atkin *et al.* 2000b); *Pinus banksiana* (Tjoelker *et al.* 2009)] as the sensitivity of respiration to temperature, measured by Q_{10}, is correlated positively with E_o values. A rapid decline in activation energy of respiration during late winter and early spring without significant changes in soil water content is observed in concert with a higher rate of respiration. This is likely associated with the changes in developmental stages of the grass and kānuka due to high growth and re-allocation of resources with spring budburst and flowering. This finding is in agreement with previous study of seasonal variation and temperature effects on the sensitivity of respiration activity in pistachio trees (*Pistacia vera*), which increased throughout the transition from immature to mature tissues (Marra *et al.* 2009). Moreover, the seasonal changes in E_o values observed in the field were neither related to instantaneous changes in soil water content nor to seasonal changes in substrate availability within both plant species. The lack of association between the temperature dependence of leaf respiration and substrate availability is also observed in several other studies (Zaragoza-Castells *et al.* 2008; Rodríguez-Calcerrada *et al.* 2010; Searle *et al.* 2010). Therefore, it is not possible to fully disentangle the combined effects of temperature, soil water content and plant developmental stages on respiration. However, Atkin and Tjoelker (2003) suggest that Q_{10} (here equivalent to E_o) of plant respiration is higher when respiration is limited by enzymatic capacity than when respiration is limited by substrate supply.

3.4.6 Response of respiration to temperature and soil water content

When the long-term seasonal variability in respiration was described by the mixed-effects model (Equation 2.6) with leaf temperature and soil water content, the resulting R_{10} values observed in the field were higher than those in the experiment in controlled conditions (Chapter 2). Similarly, the E_o values were also found to be higher in the field conditions than those in the controlled environment experiment. Values of θ_c were found to be different for the grass and kānuka under similar growth conditions. R_{10} values for the grass were found to be very sensitive to soil water content when compared to values for kānuka. R_{10} values of the grass started declining when θ values fell below nearly 50 % where as in kānuka, R_{10} values declined when θ values fell below 26 %. In contrast, it is likely that E_o values increase with decreasing soil water content since the R_{10}-E_o relationship is the same in both plant types. The sensitivity of θ values was more sensitive in both species in response to long-term changes in soil water content when compared to the short-term changes in soil water content under controlled growth cabinet conditions. These differences in the sensitivity to θ values observed in the field are likely associated with differences in the water requirements to meet growth demands under varying seasons.

The impacts of seasonal variability in temperature on leaf dark respiration are mediated by changes in soil water content, substrate availability and the developmental stages of the grass and kānuka. There is growing evidence that leaf respiration decreases with decreasing soil water content and the onset of drought may accentuate the downward adjustment of leaf respiration to hot conditions in summer (Huang *et al.* 2005; Rodríguez-Calcerrada *et al.* 2010). However, if soil water content is not limiting, growth temperature acts as the major environmental variable in regulating respiration. The impact of soil water content on the basal respiration rate of the grass and kānuka corroborates the findings of Reich (2010) who identified that during warm conditions, plants immediately dampen their temperature response by “down-shifting” their base

respiration, and then "rev up" again in response to cooling. It is very clear that soil water content is important during warmer months in regulating the rate of respiration and the apparent acclimation to changing temperatures in the field. Over the range of environmental conditions observed during these measurements, the stimulatory effect of increasing soil water content on respiration is unavoidable in carbon exchange models when considering the response of respiration to decreasing temperature.

3.4.7 Acclimation of respiration in response to multiple environmental variables

Previous studies on the short-term response of respiration to temperature have showed an immediate change in respiration rate whereas exposure to a new growth temperature for a long period may allow those plants to 'acclimate' (adjusting their R_{growth} in response to changing environmental conditions) (Gunderson *et al.* 2000; Xu and Griffin 2006; Ow *et al.* 2008a; Ow *et al.* 2008b; Ow *et al.* 2010). The seasonal variation in R_{10} in the grass and kānuka (higher in winter and lower in summer), associated with a shift in the elevation of the entire temperature response function of respiration, is clear evidence of 'Type II acclimation' which is described as a temperature-mediated change in respiratory capacity (Atkin and Tjoelker 2003). Acclimation to high temperature often results in a reduction in Q_{10} (related to E_o in the present study) (Atkin and Tjoelker 2003). Even though the reduction in E_o was prominent in summer months, very low values of E_o were observed in late winter to mid-spring within both plant types. The short-term change in Q_{10} (E_o) that predominantly regulates 'Type I acclimation' is subjected to biochemical and physiological adjustment in respiration, linking plant physiological activities (e.g. leaf growth and senescence) and biochemical components. The involvement of both types of acclimation occurring in individual plants is reported in previous studies (e.g.,Xu *et al.* 2007; Armstrong *et al.* 2008). Xu *et al.* (2007) quantified the relative involvement of type I and type II acclimation from the slope of the regression line fitting R_{10}–E_o relationship. In this study, I have

identified a significant type II acclimation in kānuka (less steep relationship) while a predominant type I acclimation in the grass (steeper relationship) (Figure 3.18). This species-specific association between R_{10} and E_o in regulating the thermal acclimation of respiration is likely linked to an interactive influence of metabolic activities involving changes in substrate availability, ATP demand, respiratory pathways, respiratory enzyme capacity or adenylate limitations in response to long-term changes in environmental conditions.

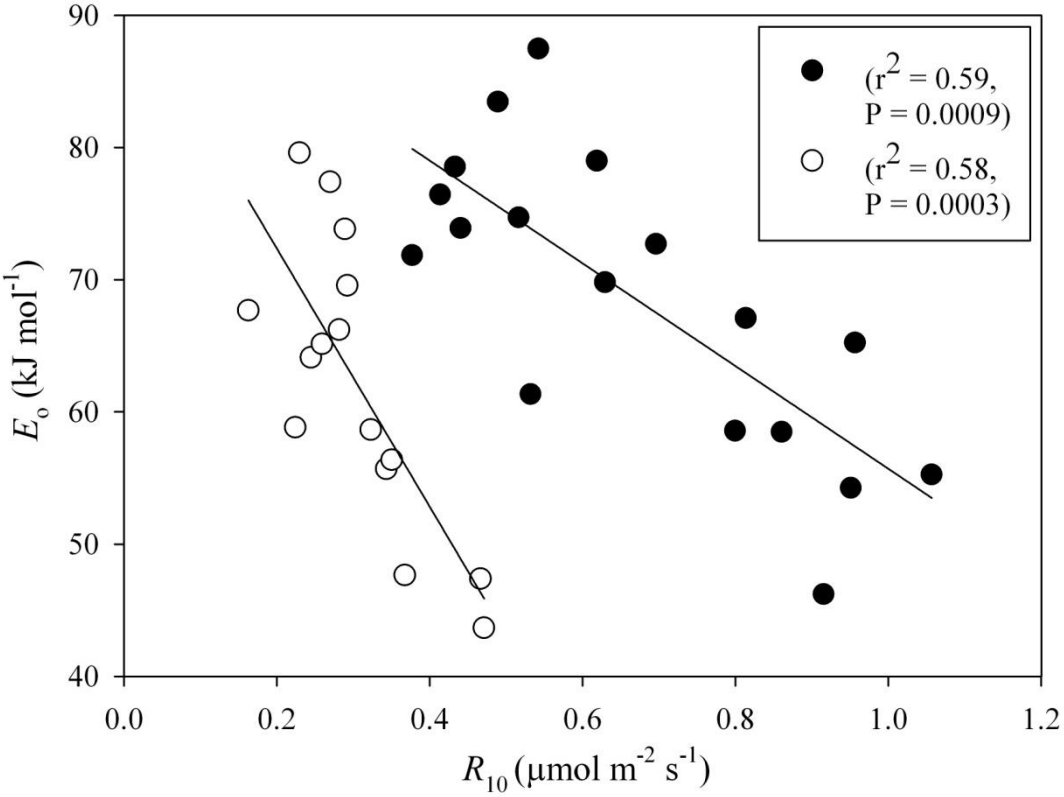

Figure 3.18 The relationship between R_{10} and E_o is fitted with a linear regression relationship for kānuka (closed circles) and the grass (open circles), r^2 and P values are shown for both plant types.

Previous studies have identified that the degree of acclimation differs widely among species (Tjoelker *et al.* 1999; Atkin and Tjoelker 2003; Atkin *et al.* 2005a; Atkin *et al.* 2005b). It has also been recognised that acclimation reduces the extent of thermal response of respiration over longer periods (Atkin *et al.* 2000b) and therefore it is critical to analyse the time needed for a complete respiratory acclimation is achievable in these plant types. Unlike other seasonal or

temperature response studies, acclimation did not occur in this study in response to temperature changes within a short duration (1-7 days) (Atkin *et al.* 2000b; Bolstad *et al.* 2003; Lee *et al.* 2005; Armstrong *et al.* 2006b; Atkin *et al.* 2006b; Armstrong *et al.* 2008; Ow *et al.* 2008a; Ow *et al.* 2008b; Ow *et al.* 2010), and perfect respiratory acclimation (homeostasis) was not observed in either plant type. However, respiration at a basal reference temperature of 10 °C (R_{10}) correlated significantly ($P < 0.05$) and negatively with field temperature averaged over 40-150 days, with a peak correlation using a 125-day temperature window, resulting in a downward shift of the entire short-term temperature response function that is consistent with previous studies (Atkin and Tjoelker 2003; Atkin *et al.* 2005b; Ow *et al.* 2008a; Ow *et al.* 2008b). Several previous studies have found leaf respiration to acclimate to changes in temperature that is typically calculated over a short time window (1-7 days) of averaged temperature (Atkin *et al.* 2000b; Lee *et al.* 2005; Xu and Griffin 2006; Tjoelker *et al.* 2009; Ow *et al.* 2010). In this study, although a small peak at day 1 (for the grass) and day 2 (for kānuka) was found in the correlation (r^2 value) between respiratory parameters and temperature, the strongest correlation occurred between 100 and 150 days in each analysis (Figure 3.13). Searle *et al.* (2010) reported respiration in two species of *Chionochloa* (tussock grasses) to acclimate to changes in temperature over seasonal but not short timescales. It is possible that the slow acclimation observed in *Chionochloa* spp. as well as in kānuka and grass was complicated by seasonal signals such as changes in daylength.

The rapidity of thermal acclimation has been reported to vary from less than one week (mostly under controlled conditions) to several weeks (under field conditions) (Atkin *et al.* 2000b; Bolstad *et al.* 2003; Armstrong *et al.* 2008; Ow *et al.* 2008a; Ow *et al.* 2008b; Ow *et al.* 2010). Partial respiratory acclimation to new growth temperature occurred in a deciduous angiosperm *Populus deltoides* var. *nigra* x *canadensis*, (Ow *et al.* 2008a) and in an evergreen conifer *Pinus radiata*, (Ow *et al.* 2008b), after a week, but full acclimation did not occur in these

species for several weeks until the appearance of new leaves. Results of the present study showed a strong correlation between R_{10} and previous 125-day temperature and previous 60-day soil water content as well as between E_o and previous 150-day temperature and previous 125-day soil water content. It is interesting to note that respiratory acclimation to seasonal changes in soil water content was apparently faster than to seasonal changes in temperature. With respect to the rate of fluctuation, it is likely that variation in soil water content is relatively gradual when compared to the temperature variations that may subsequently alter the response mechanism of the plants. Slow rate of acclimation has also been identified in *Chionochloa* spp. that are exposed to frequent, extreme temperature changes in the alpine zone of New Zealand (Searle *et al.* 2010). This "slow" acclimation to both soil water content and temperature may be an adaptive plant mechanism to increase the energy efficiency of construction costs in highly variable environments when environmental conditions change rapidly. (2010). So paradoxically, the timing of acclimation may depend on the stability of environmental conditions (i.e., frequent variations tend to slow down rates of acclimation while fairly stable conditions or gradual variations may increase the rate of acclimation).

Similar to R_{10} values, E_o values did not show a significant correlation with the growth temperature, i.e., minimum temperature experienced by the plant on the day preceding measuring day or instantaneous soil water content in the grass or kānuka. However, as the temperature and soil water content response varied throughout the year, a strong positive relationship between E_o and 100-250 day previous minimum temperature was found in both plant types with a peak correlation at 150-days. In addition, a strong negative relationship between E_o and previous 60-200 days soil water content was found in both plant types with a peak correlation at 125 days. These both support respiratory acclimation to long-term changes in temperature and soil water content. It is noteworthy that the thermal acclimation of activation energy is exemplified by a thermal response that is distinctly different when instantaneous and

seasonal comparisons are made (Figure 3.12). Therefore, it is clear that short-term changes in growth temperature may alter the rate of respiration, but exposure to a growth temperature for a long period results in thermal acclimation (Atkin and Tjoelker 2003; Atkin *et al.* 2005a; Atkin *et al.* 2005b).

Respiratory acclimation in kānuka and the grass to seasonal changes in growth temperatures was greater than photosynthetic acclimation potential. This finding supports the results of other studies (Medlyn *et al.* 2002b; Ow *et al.* 2008a; Ow *et al.* 2008b; Warren 2008; Way and Sage 2008b; Dillaway and Kruger 2010; Ow *et al.* 2010) reporting that respiratory acclimation is more pronounced than that of photosynthetic acclimation in many higher plants. The process of respiratory acclimation is complicated not only by environmental variables such as temperature and water availability, but also by carbohydrates, the product of photosynthesis. Thus, this dual study on both respiration and photosynthesis in plants subjected to changing conditions in the field enhances our understanding of the interactions between these two processes.

3.4.8 Balance between respiration and photosynthesis

Even though photosynthesis and respiration are interdependent (Whitehead *et al.* 2004a), a change in growth conditions will alter the rate of photosynthesis and respiration. This results in variation in the R_{growth}/A_{max} ratio because the temperature sensitivity of these processes are different (Dewar *et al.* 1999; Gifford 2003) and the degree of variation is determined by the temperature coefficient of each process (Atkin *et al.* 2007). Results from the present study show that the R_{growth}/A_{max} ratio differed in the grass and kānuka and it was relatively high in late winter and early spring for kānuka, while it was high in warmer months for the grass. The higher values of R_{growth}/A_{max} in kānuka during colder months are due to the higher rate of respiration whereas in the grass they are due to relatively low rates of photosynthesis during warmer months. This

result is comparable with the findings of Ow *et al.* (2010), suggesting that homeostasis of R_{growth}/A_{max} is not often achievable when thermal acclimation of photosynthesis and respiration are low. The R_{growth}/A_{max} ratio may also vary with respect to changes in soil water content. Reducing soil water content and increasing growth temperature tended to result in high R_{growth}/A_{max} ratios in the grass [which is comparable with the results of others (Flexas *et al.* 2006; Atkin and Macherel 2009)]. Kānuka showed a contrasting trend, with high R_{growth}/A_{max} ratio in winter and early spring and low R_{growth}/A_{max} ratio in summer due to temperature regulated variations in photosynthetic rate. This indicates that R_{growth}/A_{max} ratio is likely to be species dependent, as the optimum level of temperature and soil water content and temperature sensitivity vary among species. As a result, it is important to identify the factors regulating photosynthesis and respiration in plant species in order to accurately model the influence of environmental parameters on carbon gain and carbon loss within an environment undergoing changing climatic conditions.

3.5 SUMMARY

The results of the present study demonstrate that photosynthesis and leaf respiration in a grass and a shrub are sensitive to seasonal changes in temperature and soil water content. Photosynthetic parameters tended to retain a relatively constant response to changes in temperature and soil water content and did not acclimate following changes in seasonal growth conditions. In contrast, respiratory parameters tended to acclimate much more strongly. Therefore, differences in thermal acclimation of respiration and photosynthesis resulted in a relatively lower rate of respiration to photosynthesis in warmer months. Even though temperature plays a major role in photosynthetic and respiratory activity of these plant types, the impact of seasonal variation in soil water content is also important because it alters the temperature sensitivity of these processes. This influences the long-term response of

photosynthesis and respiration to environmental variables. Importantly, the impact of low soil water content on respiration is to reduce its apparent sensitivity to temperature. This may effectively decrease the rate of respiration during warmer summer months, thereby enhancing "apparent" thermal acclimation. Finally, these findings suggest that when modeling photosynthetic and respiratory processes, it is important to consider the influence of soil water content conditions in the temperature response of photosynthetic and respiratory model parameters, in addition to other major environmental and plant-related factors. For this reason, the next chapter will investigate the impact of incorporating seasonally–mediated changes in photosynthesis and respiration in response to changes in temperature and soil water content in a model to predict net carbon exchange in a pasture-shrub system.

CHAPTER 4

Modelling the response of canopy carbon exchange to seasonal changes in environmental variables in a regenerating shrubland

Chapter 4

4.1 INTRODUCTION

Models that operate at leaf levels can provide the physiological basis for scaling-up gas exchange measurements from the leaf to canopies. Such models are driven by climate variables (e.g. temperature, rainfall, irradiance) and employ algorithms to simulate important biophysical processes including photosynthesis, respiration and allocation of carbon, the exchange of soil water to the atmosphere, and the decomposition of organic matter and nitrogen dynamics in the soil. Such process-based models are used generally for investigating the influence of environmental variation on plant carbon exchange. Moreover, these models serve as effective tools to assess the impacts of climate variability and its long-term effects on carbon dynamics to extrapolate measured carbon exchanges from individual sites to regional and global scales (Wohlfahrt *et al.* 1998; Yuan *et al.* 2008). There have been many reports about different models, each with its own set of assumptions and generalisations (Ryan *et al.* 1996b; Mäkelä *et al.* 2000; Waring and McDowell 2002; Sitch *et al.* 2003; Ito *et al.* 2005; Medlyn *et al.* 2011). Since respiration and photosynthesis are dependent on changes in temperature, the temperature responses of these processes are usually incorporated. However, the influence of soil water content on these physiological processes is often not well explained. Recent developments in net carbon exchange using eddy covariance measurements show that the exchange of carbon dioxide in forest ecosystems is strongly influenced by soil water content, direct and diffuse light levels and temperature (Malhi *et al.* 2002; Dunn *et al.* 2007; Misson *et al.* 2007). So it is critical to incorporate soil water availability as a driving variable in process-based carbon balance models.

To describe photosynthetic responses to environmental variables, several modelling studies have used the biochemical model of photosynthesis (Farquhar *et al.* 1980) and demonstrated how photosynthetic rates vary among species (Wullschleger 1993; Kattge and Knorr 2007; Kattge *et al.* 2009). Recent studies have identified the acclimation of photosynthesis

to changes in temperature and carbon dioxide (Hikosaka *et al.* 1999; Medlyn *et al.* 2002a; Ellsworth *et al.* 2004; Kattge and Knorr 2007), but relatively little is known about the acclimation of photosynthetic parameters to other changes in climate. Acclimation to changes in climate involves a combination of different stresses that may not be simply the sum of the components (Mittler 2006). Moreover, recent studies have demonstrated that the impacts of a combination of different stress conditions are different from individual stress conditions (Illeris *et al.* 2004; Mittler 2006; Centritto *et al.* 2011). To date, only few investigations have attempted to analyse the acclimatory response of photosynthesis with a combination of varying environmental conditions.

Respiratory acclimation has the potential to alter the size of ecosystem carbon pools (Wythers *et al.* 2005) and has important implications for predictions of plant respiration in response to climate change (King *et al.* 2006). The acclimation response can be substantial and rapid and have a significant effect on rates of above ground net-primary production (Wythers *et al.* 2005). Several field studies have reported thermal acclimation in response to seasonal changes at varying time-scales (Atkin *et al.* 2000b; Xu and Griffin 2006; Ow *et al.* 2010; Searle *et al.* 2010). However, such acclimation is not yet included in global models of carbon cycle responses and feedback to climate, even though it is common in a wide range of plant species (Loveys *et al.* 2003; Campbell *et al.* 2007).

Since changes in ambient temperature and soil water content occur in the field conditions simultaneously, it is important to understand the impact of the combination of these conditions on physiological acclimation and the impact on changes in annual net carbon exchange. Climate change is expected to alter forest productivity in the future and models are used widely to estimate the combined effects of varying factors affecting forest productivity (Medlyn *et al.* 2011). The timing and amount of water availability during different seasons may exert significant variations in grass productivity (Knapp and Smith 2001; Knapp *et al.* 2002; Fay *et al.*

2011). Previous studies have emphasised the importance of using a coupled-model approach with seasonal variations to understand forest carbon and water balances (Kosugi *et al.* 2003; Zhu *et al.* 2011). Zhu *et al.* (2011) showed that in order to parameterise photosynthetic parameters in response to seasonal variations, it is essential to incorporate the kinetic properties of Rubisco, temperature dependence of photosynthetic parameters and the seasonal trends in stomatal conductance parameters in coupled-models. However, little is known about the response of aboveground physiological processes to seasonal changes including acclimation, phenological changes, nutrient interactions, etc (Hanson and Weltzin 2000; Wilson *et al.* 2000; Medlyn *et al.* 2002b; Eamus 2003; Xu and Baldocchi 2003; Brando *et al.* 2006).

Seasonal change is not merely a change in growth temperature, but involves changes in wind, rainfall and light levels. Seasonal variation in the Canterbury region is very distinct with abrupt day-to-day fluctuation in growth temperature that is often accompanied by changes in soil water content. Brown *et al.* (2009) observed a wide range of root-zone soil water content due to inter-annual variability in rainfall pattern. However, the observed range of soil water content was not too large to induce severe soil water deficit for a prolonged period in this region.

In this chapter, a modelling approach was used to understand the response of annual leaf carbon exchange in a grass and kānuka to two varying environmental variables (growth temperature and soil water content). The objective of this study was to examine the role of the net ecosystem exchange model (NEEMo) for assessing above-ground canopy carbon exchange, which integrates the effects of photosynthesis and dark respiration and takes into account the influence of physical characteristics of the site (including soil water content) and the presence of thermal acclimation within multiple layers in a canopy. The model was used for the following five main aims: (1) to understand the daily and annual canopy carbon exchange of the grass and kānuka in response to seasonal variations in environmental variables (2) to analyse the sensitivity of the model to changes in photosynthetic and respiratory parameters (3) to compare

estimates of annual net canopy carbon exchange using a static (instantaneous parameters) and a variable (seasonal parameters) approach as described by Whitehead *et al.* (2004a); (4) to compare carbon exchange estimates of the grass and kānuka using the temperature response of photosynthetic parameters and Q_{10} values of respiration observed in the field and under similar growing conditions in the controlled growth cabinet; (5) to predict the changes in net canopy carbon uptake under varying climate change scenarios. Hourly outputs of the model were summed to give daily, monthly and annual values. This coupled model is ideal for estimating the seasonal changes in carbon exchange since it is mainly driven by meteorological variables that are often readily available. Therefore, a distinctive feature of this study is linking plant physiological measurements with environmental variables to quantify canopy carbon exchange for a two-layer system comprising kānuka shrubs and grassland. Another objective was to predict net ecosystem exchange of a pastoral ecosystem reverting to shrubland, under different climate change scenarios.

4.2 MATERIALS AND METHODS

4.2.1 Model description, simulation and validation

The Net Ecosystem Exchange Model (NEEMo) is a one dimensional, multi-layered, process-based model designed to link the measured carbon exchange processes of ecosystem components and to predict carbon balance in relation to climate variables and water balance (Whitehead and Walcroft 2005). The model integrates site variables and the components (kānuka and the grass) with water balance. The weather data recorded at the experimental site, including half-hourly measurements of relative humidity (Vaisala HMP50, Helsinki, Finland) and half-hourly and daily measurements of maximum and minimum air temperatures (Vaisala HMP50, Helsinki, Finland), short-wave radiation (Model LI-190, Li-Cor BioSciences, Lincoln, NE, USA) and rainfall (Texas raingauge, Model TR-525M-R1, Texas electronics, USA) are required for

running the model. The seasonal variation in daily maximum and minimum air temperatures, radiation, rainfall, and vapour pressure deficit is demonstrated in Figure 4.1.

The model couples a photosynthesis-stomatal conductance model for leaves (Leuning 1995) with a simple water balance model (Whitehead *et al.* 2001), and scales-up the leaf level measurements of photosynthesis and respiration to the canopy level using submodels for radiative transfer, energy balance, evaporation and photosynthesis, in combination with a site water balance model to incorporate the effects of seasonal root-zone water deficit on canopy carbon uptake and transpiration. Among various carbon exchange processes, photosynthesis and respiration by foliage regulate the net carbon exchange between the vegetation and the atmosphere. These processes are strongly regulated by environmental variables. Therefore, linking these processes with the climate variables allows me to interpret the carbon flux of above-ground components and to estimate the long-term carbon exchange of canopies in relation to climate.

The canopy is divided into 10 horizontally homogenous layers, with the top five layers for kānuka and bottom five layers for the grass. An elliptic leaf angle distribution of foliage is assumed for the calculation of radiative transfer through the canopy for sunlit and shaded foliage in both canopies, including foliage clumping and gaps and interception by the stems and branches. This requires the estimates of leaf area, branch surface area, and the degree of clumping to represent the spatial distribution of foliage. Other site variables are the fraction of rainfall intercepted by the canopy, and the root-zone depth and the root-zone water holding capacity.

Photosynthesis (A) is limited by the minimum of two processes and the parameters describing these processes are given in Chapter 2. Values for V_{cmax} and J_{max} at the top canopy of kānuka and the grass, considered as layer one of the component, were calculated from the A/C_i response curves (see Chapters 2 and 3) at a range of temperatures.

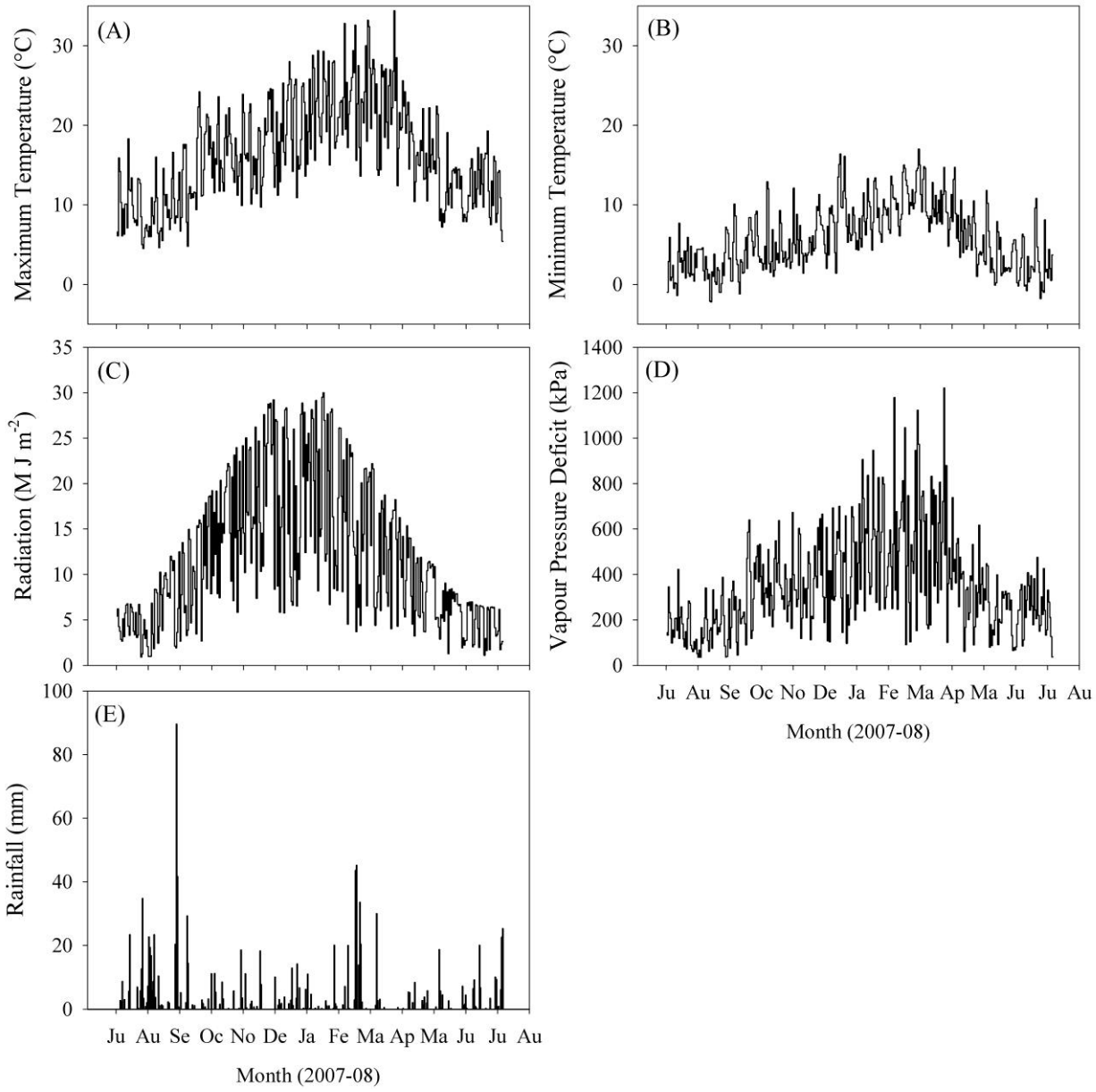

Figure 4.1 Seasonal variation in maximum and minimum temperatures (A and B), short-wave radiation (C), soil water content (E) and rainfall (F) measured at the experimental site. Modelled seasonal variation in vapour pressure deficit (D) is shown from July 2007 to June 2008.

The coupled photosynthesis – stomatal conductance model for individual leaves is taken from Leuning (1995), where the rate of photosynthesis, following Farquhar *et al.*, (1980) and von Caemmerer and Farquhar (1981), is given as:

$$A = \min\{A_c, A_q\} \quad \text{..... (4.1)}$$

Where A_c is the rate of photosynthesis limited by the rubilose 1,5, bisphosphate (RuBP) carboxylation activity of the enzyme ribulose 1,5-bisphosphate carboxylase-oxygenase (rubisco), A_q is the rate limited by RuBP regeneration by the electron-transport system and min { } refers to the minimum of the two rates. Equations explaining the rate of photosynthesis limited by rubisco activity and regeneration of RuBP are described in Chapter 2 (Equation 2.1 & 2.2, respectively). NEEMo integrates a radiation transfer model (incorporating direct and diffuse components of radiation in PAR, NIR and thermal wavebands) for estimating the leaf energy balance to calculate photosynthesis for sunlit and shaded foliages in each layer (Leuning *et al.* 1995). Total photosynthesis is estimated across layers within the canopy and daily values are obtained using Gaussian integration following Goudriaan & van Laar (1994).

When *A* is limited by RuBP regeneration,

$$A = J\frac{Cc - \Gamma *}{4Cc + 8\Gamma *} - \mathrm{Rd} \qquad \text{..... (4.2)}$$

Where J is the rate of electron transport C_c is the CO_2 partial pressure at Rubisco, Γ*is the photorespiratory compensation point, R_d is respiratory CO_2 release other than by photorespiration (day respiration) and is presumed to be primarily mitochondrial respiration.

The response of electron transport rate (*J*) at a given irradiance (*Q*) was calculated from the measured rate of photosynthesis and the response of *J* to *Q* is described using a non-rectangular hyperbola, by rearranging Equation (4.3) from Sharkey *et al* (2007) as:

$$(\alpha Q + J_{\max} - \sqrt{((\alpha Q + J_{\max}{}^2) - 4\,\beta\alpha Q J_{\max})})/2\beta = 0 \text{..... (4.3)}$$

Where J_{max} is the maximum rate of electron transport at saturating irradiance, β is the convexity of the hyperbola and α is the quantum efficiency of the electron transport.

The average values of convexity (β) and the apparent quantum efficiency (α) of electron transport (or the initial slope of the *J*/*Q* response curve) were incorporated into the stomatal model (Leuning 1995).

The intercellular partial pressure of CO_2, c_i, is calculated from the partial pressure at the leaf surface, c_s, and the stomatal conductance to CO_2 transfer, g_{sc}, where:

$$c_i = c_s - (A / g_{sc}) \quad \text{..... (4.4)}$$

The coupling of photosynthesis and stomatal conductance and the relationship with c_s and air-saturation deficit at the leaf surface, D_s, is modified from Leuning (1995) as:

$$g_{sc} = g_{sc0} + \frac{\tau\, a\, A}{(c_s - \Gamma)(1 + [D_s - D_{smin}]/D_{s0})} \quad \text{..... (4.5)}$$

Where g_{sc0} is the residual conductance at the light compensation point, D_{s0} describes the sensitivity of g_{sc} to D_s, D_{smin} is the minimum value, Γ is the CO_2 partial pressure at compensation in the presence of photorespiration of air saturation deficit below which g_{sc} remains at its maximum value and a is related to the intercellular CO_2 partial pressure. The coefficient τ decreases g_{sc} when daily soil water storage falls below a maximum value.

The simple daily water balance model (Whitehead *et al.* 2001) calculates the soil water storage on the first day, W_i, using the following water balance equation.

$$W_i = W_{i-1} + P_i - E_{tl} - E_{wl} - E_{ul} - F_i \quad \text{..... (4.6)}$$

Where P_i is the daily rate of rainfall, E_{ti} is transpiration from the dry plant canopy, E_{wi} is evaporation from the wet canopy, E_{ui} is evaporation from the understorey and soil and F_i is drainage from the root-zone. Surface run-off is assumed to be zero and E_{wi} is assumed to be a constant fraction of rainfall (= 0.05 P_i) (D. Whitehead, personal communication).

If W_{max} and W_{min} are the maximum and minimum soil water storage, τ in Equation (4.5) is set to 1 while $W = W_{max}$, and decreases linearly to zero when $W=W_{min}$ (Wang and Leuning 1998). Drainage from the root-zone occurs when $W_i > W_{max}$.

Net carbon uptake is modelled from the measurements of photosynthesis and respiration and stomatal conductance in the canopy following the standard procedure described in Whitehead *et al.* (2004b). The temperature dependence of photosynthetic parameters is described by following Bernacchi *et al.* (2001), as shown in Table 4.1, is integrated with the gas exchange model of Leuning (1995).

Table 4.1 Values of the parameters used for estimating the temperature response of photosynthesis according to Bernacchi *et al.*, (2001), where γ_0 is the value of the CO_2 partial pressure at compensation in the absence photorespiration (Γ^*) at a reference temperature, γ_1 and γ_2 are empirical coeffecients, K_{c0} and K_{o0} are the values of the Michaelis constants for CO_2 (K_c) and O_2 (K_o), respectively, at a reference temperature, and H (K_c) and H (K_o) are the energies of activation for K_c and K_o, respectively. The reference temperature used in the calculation was 20 °C (293 K).

Parameter	Value	Units
γ_0	0.0000326	mol mol^{-1}
γ_1	0.0571	---
γ_2	0.001321	---
K_{c0}	0.000223	mol mol^{-1}
K_{o0}	0.216	mol mol^{-1}
H (K_c)	85923	J mol^{-1}
H (K_o)	37990	J mol^{-1}

The values of slope parameters in the stomatal model, minimum stomatal conductance, and minimum air saturation deficit and sensitivity of stomatal conductance to air saturation deficit for kānuka were obtained from Whitehead *et al.* (2004b), and for the grass from other published sources (Table 4.2).

Table 4.2 List of parameters derived from measurements and used in the model to estimate annual net carbon uptake at the field site. Values of α and β represent the means of quantum yield of electron transport and convexity of the light response curve. D_{s0} is the sensitivity of stomatal conductance to D, D_{smin} is minimum value of D for decreasing g_{sc}, a_1 (g_{sc}) is stomatal sensitivity related to intercellular CO_2 partial pressure (c_i), g_{sc0} is residual stomatal conductance to CO_2 transfer and l is leaf dimension.

Parameter	Kānuka	Source	Grass	Source	Units
α	0.16	Current study	0.31	Current study	mol mol^{-1}
β	0.27	Current study	0.17	Current study	
D_{s0}	1162	(Whitehead *et al.* 2004b)	5900	(Uddling and Pleijel 2006)	Pa
D_{smin}	450	(Whitehead *et al.* 2004b)	400	Whitehead (personal communication)	Pa
a_1 (g_{sc})	4.2	(Whitehead *et al.* 2004b)	7.1	(Uddling and Pleijel 2006)	--
g_{sc0}	0.01	(Whitehead *et al.* 2004b)	0.01	(Wang and Leuning 1998)	mol m^{-2} s^{-1}
l	0.003	(Whitehead *et al.* 2004b)	0.01	(Wang and Leuning 1998)	m

Respiration is sensitive to temperature and parameters linking this relationship were estimated at a range of temperatures (Chapter 3). Monthly measurements of the rate of respiration at a base temperature of 10 °C (R_{10}), was used to estimate an Arrhenius response following Turnbull *et al.* (2003) for long-term (seasonal) changes. In the short term, respiration should exhibit an exponential response to temperature. To test the effects of seasonal acclimation, monthly measurements of R_{10} values and the monthly average value of Q_{10} for the shrub and the grass were incorporated. Therefore, daily net carbon exchange is the difference between net photosynthesis during the day and respiratory losses over the following night.

Comparing predicted value of carbon exchange derived from the model with measured values provides validation of the results. It was not possible to validate carbon exchange but measured and predicted soil water content was compared to validate the water balance model.

Half-hourly and daily measurements of soil water content at three different depths (50, 100 and 300 mm) were recorded using soil water content sensors (Model ML2x ThetaProbe, Delta-T Devices, Cambridge, UK) at the experimental site. Daily measurements of soil water content recorded at a depth of 150 mm were compared with the simulated values of soil water content to validate the modelled results.

4.2.2 Sensitivity analysis of the model to canopy variables

The sensitivity of the model to photosynthetic and respiratory parameters was estimated by changing (± 5 % to ± 15 %) the estimated monthly values of maximum rate of RuBP carboxylation (V_{cmax}) and maximum rate of electron transport at saturating irradiance (J_{max}), respiration rate at a given temperature (R) and the annual quantum efficiency of electron transport (α) in both components.

4.2.3 Comparative analysis of seasonally acclimated carbon exchange with instantaneous and controlled growth cabinet measuements

The influence of different growing conditions on annual estimates of canopy gross photosynthesis (A_{can}) and canopy respiration (R_{can}) was investigated by comparing the field estimated values with the modelled values estimated under similar growth cabinet conditions by changing the photosynthetic and respiratory parameters of kānuka and the grass estimated at 30 % soil water content (see Chapter 2). Moreover, changes in canopy carbon uptake due to the influence of instantaneous and seasonally acclimated response of respiration were estimated by changing the temperature response parameters of instantaneous and seasonally acclimated responses of the grass and kānuka observed in the field (see Chapter 3).

4.2.4 Analysis of the response of canopy variables to predicted climate change scenarios

To determine the response of canopy photosynthesis and canopy respiration in the grass and kānuka to predicted climate change scenarios, the meteorological data required for simulation were manipulated by reducing the average rainfall up to 20 % and increasing the actual field recorded maximum and minimum temperature by 2 °C. Changes in canopy carbon exchange were analysed by comparing the response of canopy variables under different climate change conditions to estimated changes in the canopy carbon exchange under seasonally acclimated conditions. .

4.3 RESULTS

The model was run for 365 days starting from July 2007 to June 2008. The values of soil water content generated by the model following the procedure which balanced the rainfall, interception, transpiration, evaporation and drainage from the soil layer with depth up to 150 mm were tested and the observed data at a depth of 150 mm depth is plotted together with the simulated data in Figure 4.2. The model was capable of reproducing the seasonal dynamics of soil water content showing that the simulated soil water content was similar to the observed data with a coefficient of determination, $r^2 = 0.57$. The simulated values were relatively high in autumn (April-June), even though the fluctuations of seasonal variation in soil water content simulated in autumn was comparable with all other seasons.

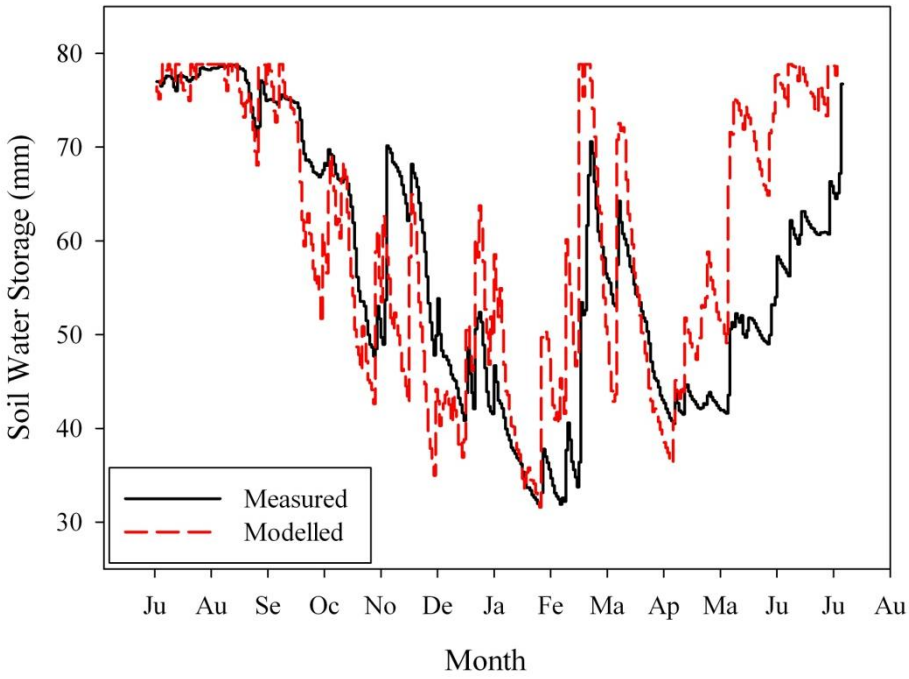

Figure 4.2 Seasonal changes in soil water storage observed (solid line) in the field and modelled (dashed line) by the net ecosystem exchange model (NEEMo) at a rooting depth of 150 mm depth from July 2007 to June 2008.

4.3.1 Seasonal changes in canopy carbon exchange

Seasonal changes in daily and annual net canopy carbon uptake were simulated for the canopy in response to changes in environmental drivers. The relative contribution of kānuka and the grass canopies to seasonal changes in daily and cumulative canopy carbon exchange are shown in Figure 4.3 and 4.4, respectively.

A high rate of canopy photosynthesis was estimated in mid to late spring for the grass, and in summer and early autumn for kānuka. Low rates of canopy photosynthesis were estimated in early to mid-winter for the grass, but in mid-winter to early spring in the shrub. Annual canopy gross photosynthesis of the ecosystem was calculated to be 26.0 Mg C ha^{-1}. The relative influence of the grass was nearly 10-fold higher than that of kānuka (annual gross photosynthesis of 23.5 Mg C ha^{-1} and 2.5 Mg C ha^{-1} for the grass and kānuka, respectively).

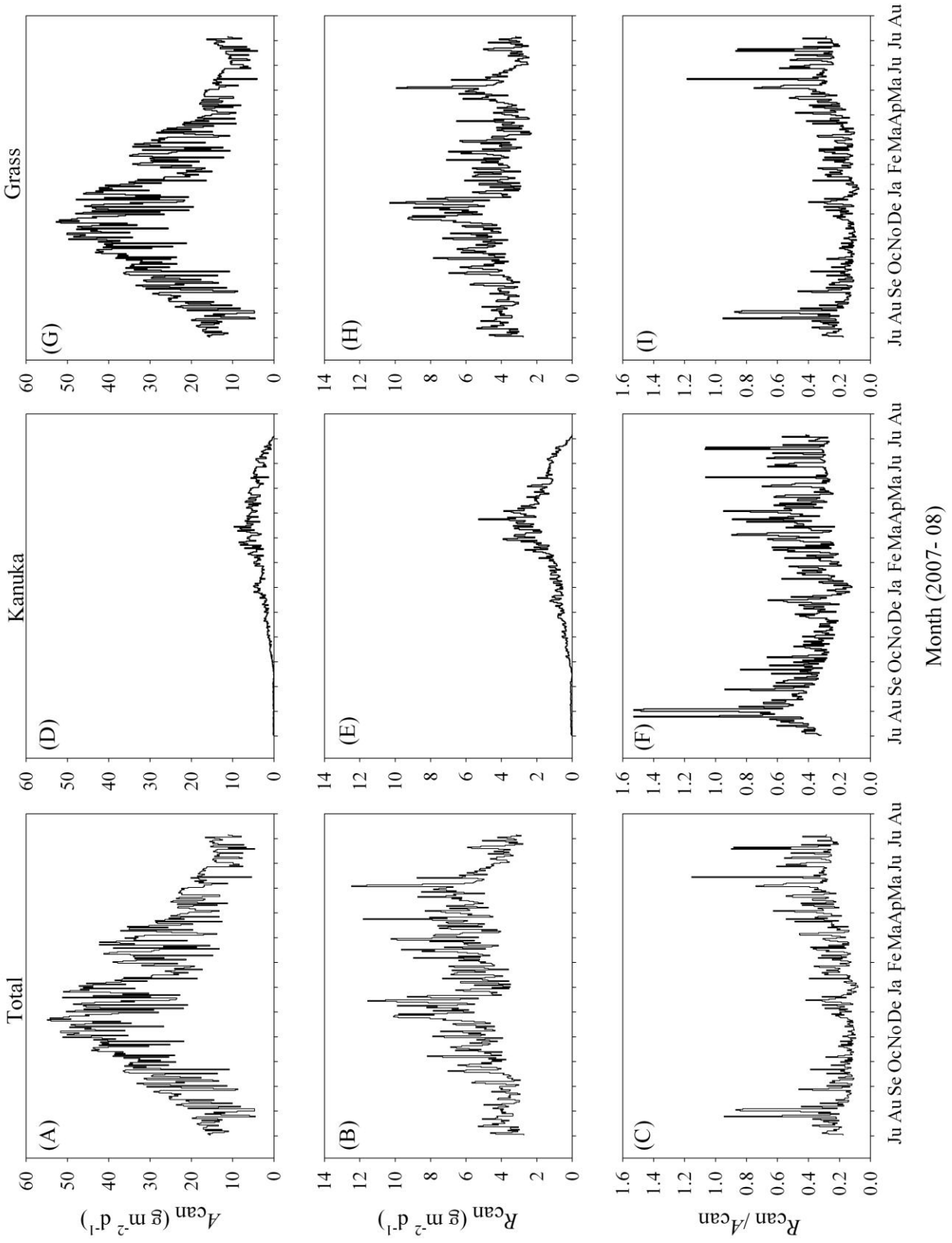

Figure 4.3 Modelled seasonal variation in daily canopy gross photosynthesis, A_{can}, (A, D, G), daily canopy respiration R_{can}, (B, E, H) and daily R_{can} / A_{can} ratio (C, F, I) for total (broken line), kānuka (solid line) and grass (dotted line) canopies at the experimental site.

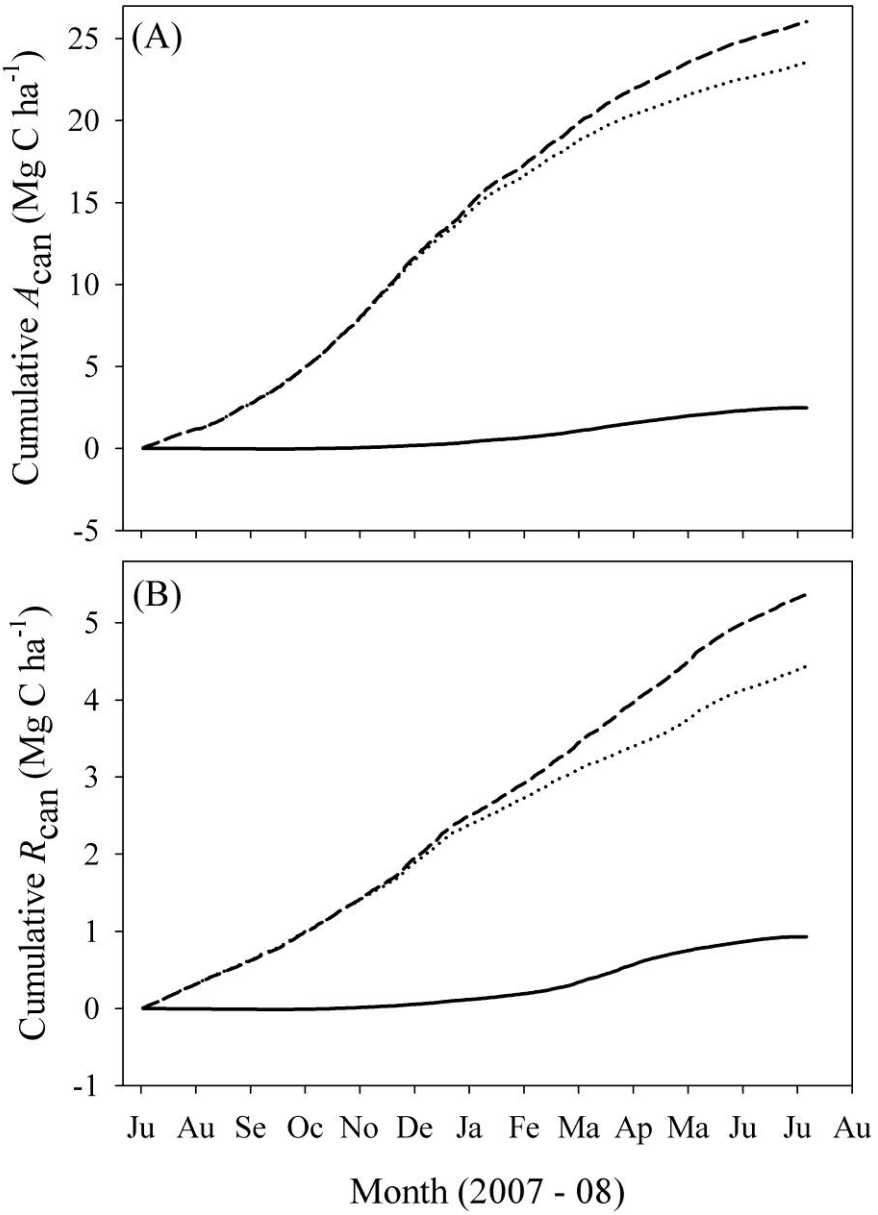

Figure 4.4 Modelled seasonal cumulative canopy gross photosynthesis, A_{can} (A), canopy respiration R_{can} (B), for total (broken line), grass (dotted line) and kānuka (solid line) canopies from July 2007 to June 2008.

Respiration was high in mid-spring and mid-autumn for the grass and then it remained relatively stable throughout the year. However, for kānuka, foliar respiration was high in mid-summer to early autumn and low in winter. Kānuka showed a relatively higher R_{can} / A_{can} ratio than that of the grass. A high R_{can} / A_{can} ratio was estimated for the grass from autumn to winter and for kānuka mainly in winter. The annual canopy respiration of the ecosystem was 5.4 Mg C

ha^{-1}. The relative contribution of the grass was nearly 5-fold higher than that of kānuka (annual canopy respiration of 4.5 Mg C ha^{-1} and 0.9 Mg C ha^{-1} for the grass and kānuka, respectively).

4.3.2 Sensitivity of the model in predicting canopy variables

To investigate the sensitivity of the model in predicting annual estimates of canopy variables, monthly measurements of photosynthetic (V_{cmax}, J_{max} and α) and respiratory (R) parameters were modified (± 5 % to ± 15 %) in both plant types and the results are shown in Figure 4.5.

Canopy photosynthesis (A_{can}) was influenced by changes in the value of α in both plant types, showing a percentage difference of -8.5 to 7.4 % between values estimated in the field and values estimated by changing α by up to ± 15 %. A_{can} was also affected by changes in V_{cmax} and J_{max} but less so than by changes in α. Changes in R of up to ± 15 % had little influence on A_{can}. Canopy respiration (R_{can}) was strongly affected by changes in R and showed a percentage difference that was directly proportional to changes in monthly values of R (± 15 %). Similar to canopy photosynthesis, changes in net canopy exchange (N_{can}) were strongly influenced (-10.7 to 9.4 %) by changes in α and to a lesser extent by changes in V_{cmax} and J_{max}. Changes in R influenced N_{can} slightly more than A_{can} but not as greatly as R_{can}. Similarly, changes in values of α positively altered canopy light use efficiency (L_{can}, -8.5 to 7.4 %) and water use efficiency (W_{can}, -5.0 to 4.2 %), and more strongly than their response to changes in V_{cmax} and J_{max} or R. Overall, changes in α influenced A_{can}, N_{can}, L_{can} and W_{can} more strongly than changes in V_{cmax} and J_{max}.

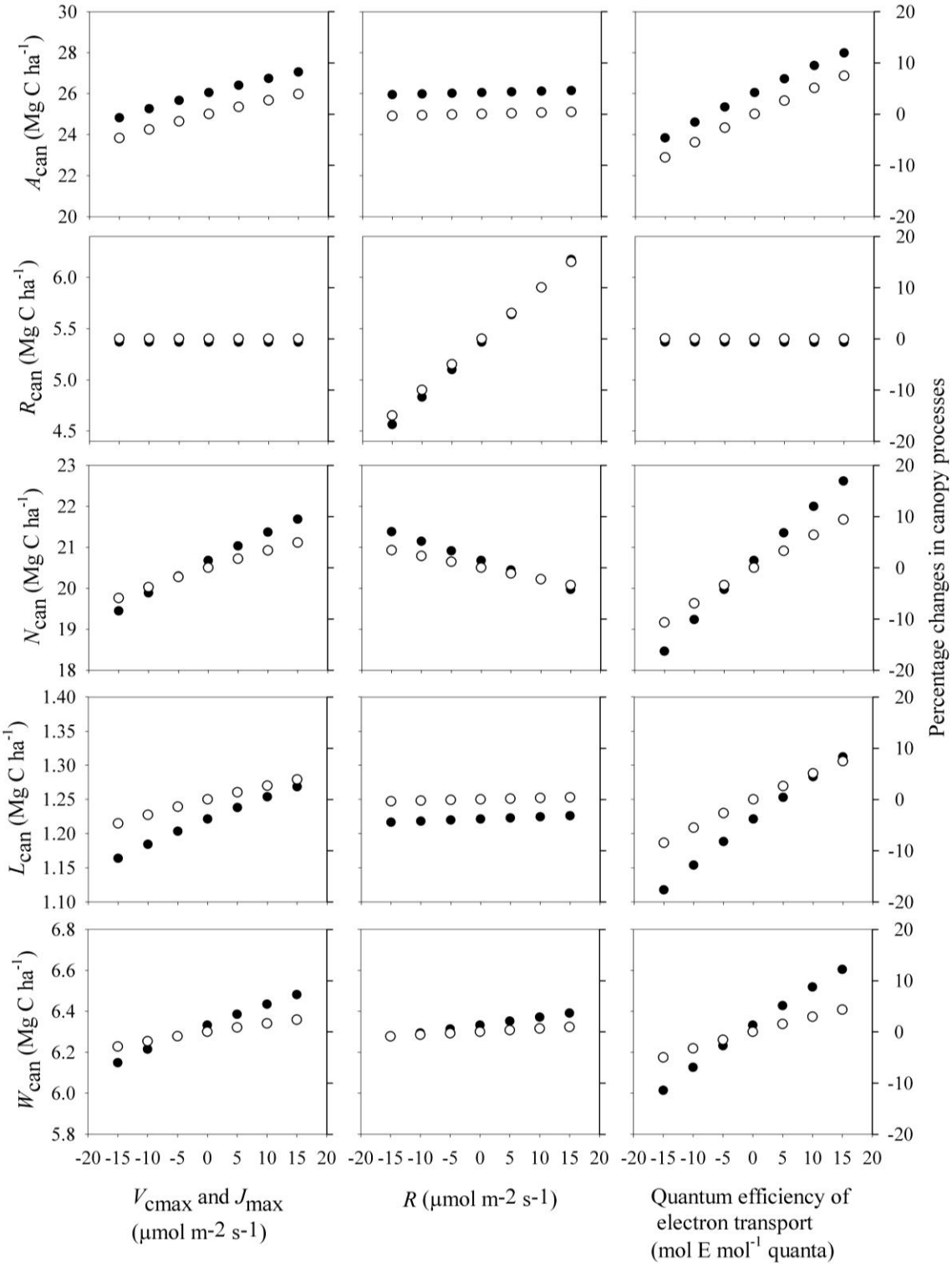

Figure 4.5 Sensitivity assessment of the model in predicting annual estimates of canopy carbon and water exchange in response to proportional changes in photosynthetic parameters (V_{cmax} and J_{max}), respiratory parameter (R) and the quantum efficiency of electron transport (α). Sensitivity to changes in parameter values for canopy photosynthesis (A_{can}), canopy respiration (R_{can}), canopy net carbon exchange (N_{can}), canopy light use efficiency (L_{can}) and canopy water use efficiency (W_{can}) are shown. Changes in the estimated values of canopy processes (closed circles) in response to proportional changes in modelling parameters and percentage changes (open circles) in the field and modified estimates are shown.

4.3.3 Comparing canopy carbon exchange under different experimental conditions

The modelled values of cumulative canopy photosynthesis (A_{can}) and canopy respiration (R_{can}), based on seasonally-acclimated parameters generated in the field experiment (Chapter 3), were found to be different from values estimated using parameters from the controlled growth cabinet experiment (Chapter 2) and instantaneous field responses (Figure 4.6).

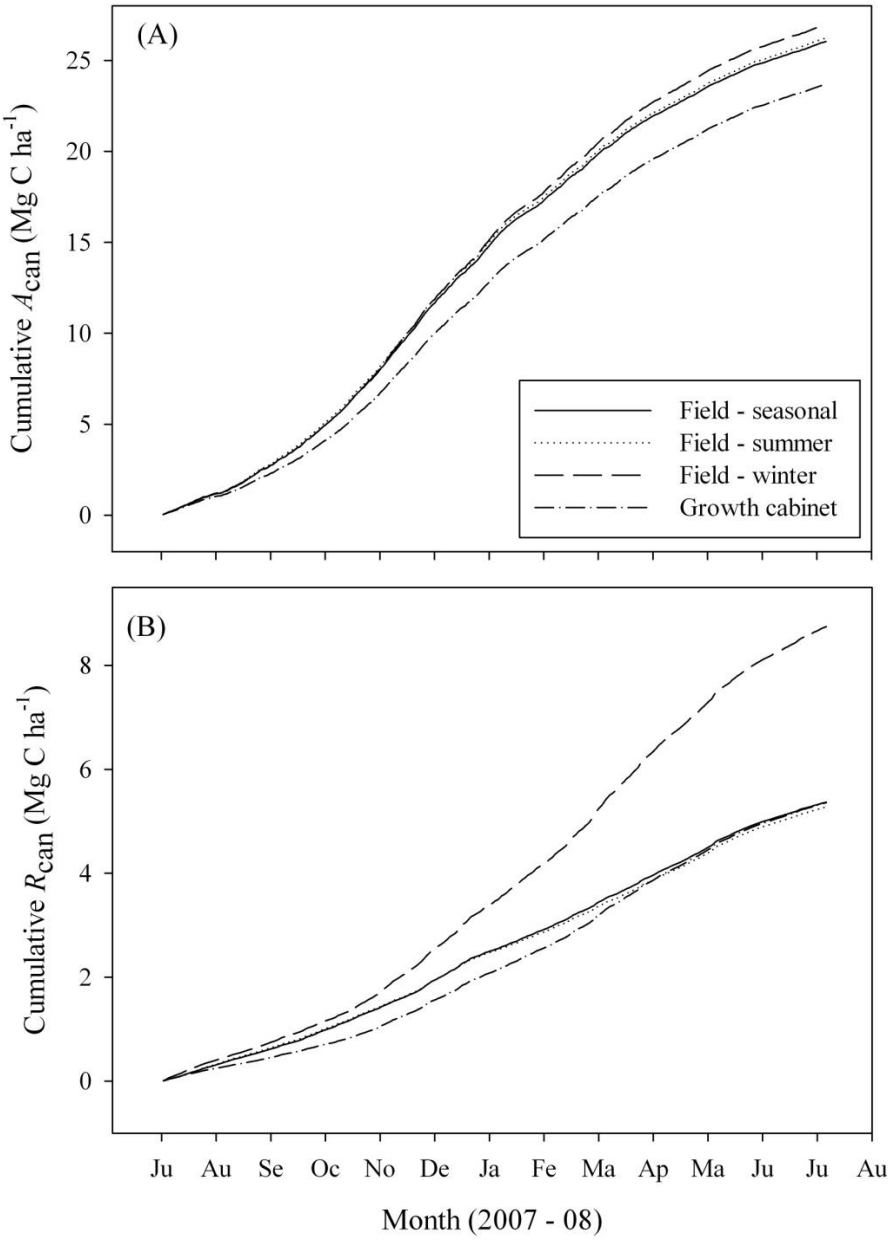

Figure 4.6 Changes in modelled seasonal cumulative canopy photosynthesis (A_{can}) (A) and canopy respiration (R_{can}) (B) using the photosynthetic and respiratory parameters observed under different experimental conditions.

The modelled cumulative value of A_{can} estimated from seasonally-acclimated responses was close to the value estimated using an instantaneous summer response, but slightly lower than that using an instantaneous winter response. The modelled value of A_{can} estimated from seasonally-acclimated parameters was much higher than the value of A_{can} estimated from the controlled growth cabinet experiment at 30 % soil water content. Cumulative canopy respiration (R_{can}) estimated from seasonally-acclimated parameters was found to be close to estimates based on an instantaneous summer response or the controlled growth cabinet based experiment at 30 % soil water content. However, R_{can} estimated from an instantaneous winter response was found to be much higher than that based on a winter or seasonally-acclimated response. To compare the changes in cumulative A_{can} and cumulative R_{can}, a percentage difference was calculated between A_{can} and R_{can} modelled under different experimental conditions compared to values obtained using a seasonally acclimated response (Figure 4.7). The cumulative value of A_{can} estimated using the growth cabinet response was nearly 10 % lower than that based on seasonally-acclimated parameters, whereas estimates based on instantaneous summer and winter values were slightly higher. The cumulative value of R_{can} estimated from a winter response was about 40 % higher than the cumulative value obtained using a seasonally-acclimated response. R_{can} estimated from growth cabinet responses or an instantaneous summer response was close to the value calculated from the seasonally-acclimated response.

4.3.4 Response of canopy variables to changes in temperature and rainfall conditions

To predict the changes in canopy carbon exchange at the experimental site under different climate change scenarios, the major climate variables (temperature and rainfall) were manipulated (Figure 4.8). The changes observed in A_{can} and L_{can} were similar in response to a reduction in rainfall of up to 20 % and an increase in maximum and minimum temperature of up to 2 °C. A reduction in rainfall of 20 % and an increase in maximum temperature of 2 °C resulted in nearly 3.5 % and 3 % reductions in A_{can}, respectively.

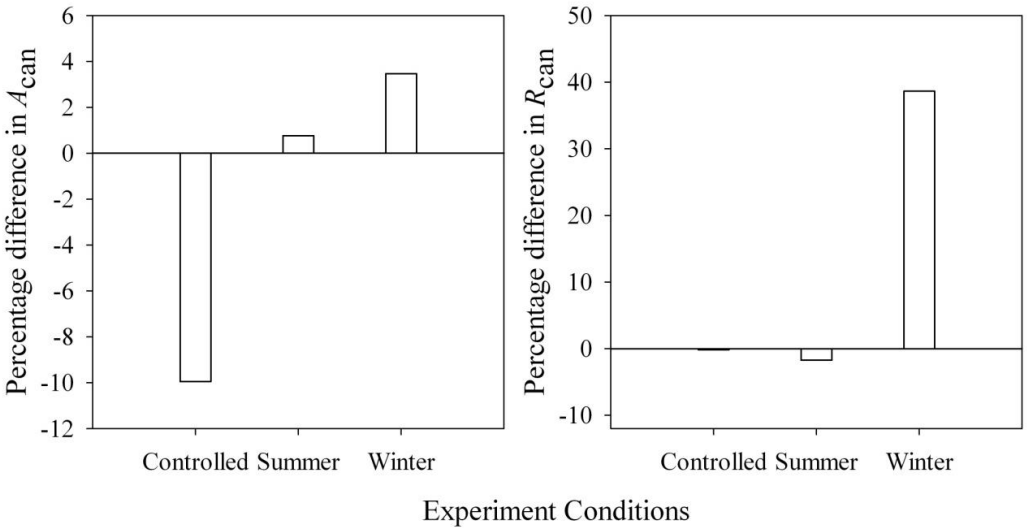

Figure 4.7 Percentage difference in annual cumulative canopy photosynthesis (A_{can}) and canopy respiration (R_{can}) modelled under different experimental conditions with respect to the seasonally acclimated field conditions, as shown in Figure 4.6

An increase in minimum temperature of 2 °C resulted in a slight increase in A_{can}. Reduction in rainfall of 20 % resulted in a slight decline in R_{can} while an increase in maximum and minimum temperatures of 2 °C had a strong impact on R_{can} (7.7 % and 6.5 % higher, respectively). Similarly, changes in rainfall and maximum temperature had a strong impact on N_{can}, which declined by up to 4.4 % and 5.8 % with a reduction in rainfall up to 20 % and an increase in maximum temperature up to 2 °C, respectively. However, an increase in minimum temperature of 2°C resulted in a slightly higher N_{can}. The changes observed in N_{can} in response to changes in rainfall and temperature conditions were similar to the responses of A_{can} and L_{can}. The influence of rainfall and maximum and minimum temperatures on cumulative canopy net carbon exchange was linked to relative changes in daily variations in vapour pressure deficit (D), canopy transpiration and soil water content simulated by the model, as demonstrated in Figure 4.9. T_{can} values decreased nearly 12 % while W_{can} values increased 10 % with a reduction in rainfall of 20 %. An increase in maximum temperature of 2°C resulted in a 7.6 % higher T_{can} and nearly 10 % lower W_{can} values. However, an increase in minimum temperature of 2°C resulted in 5 % decrease in T_{can} and 7.2 % increase in W_{can} values.

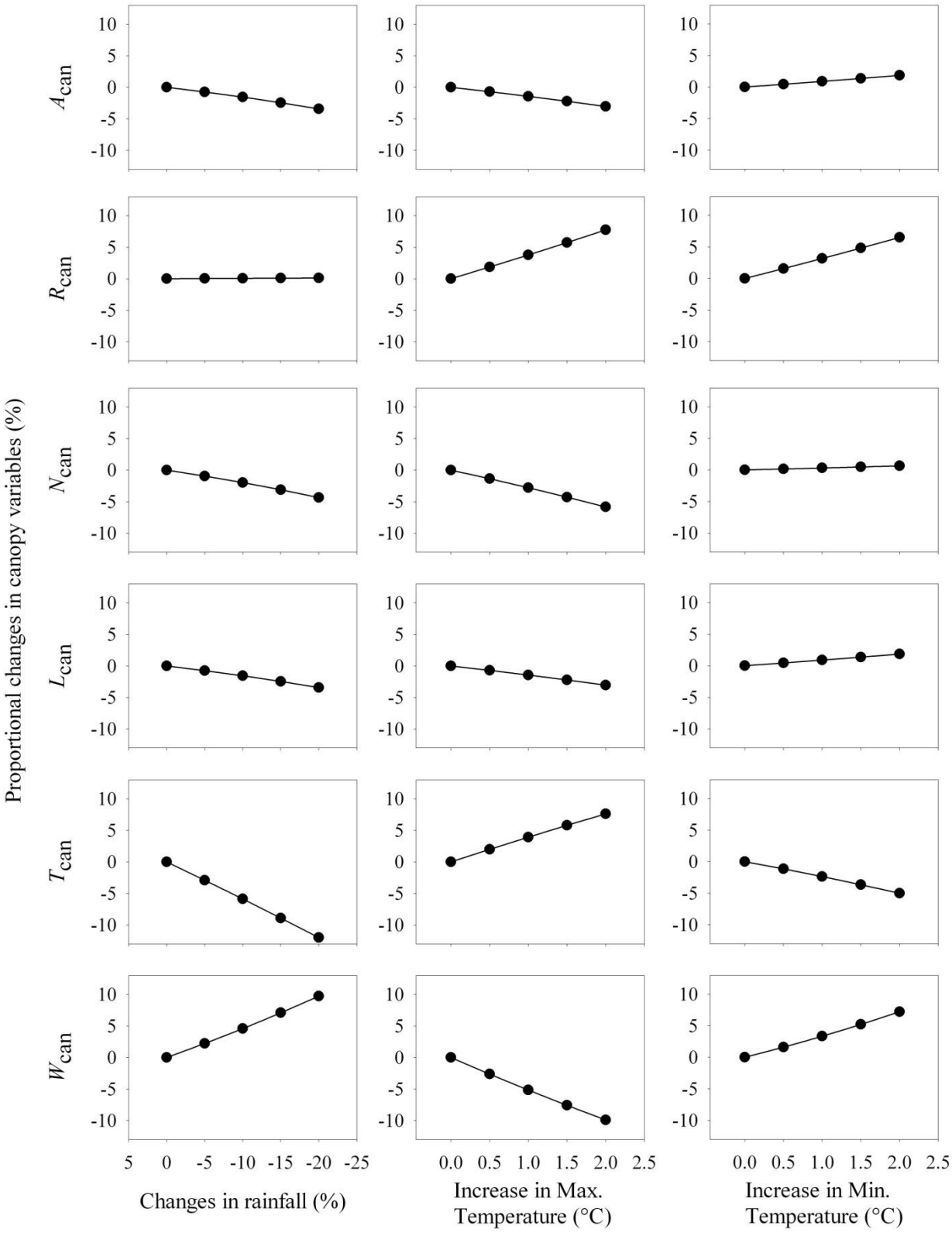

Figure 4.8 Modelled changes in the annual canopy photosynthesis (A_{can}), canopy respiration (R_{can}), canopy net carbon exchange (N_{can}), canopy light use efficiency (L_{can}), canopy transpiration (T_{can}) and canopy water use efficiency (W_{can}) in response to proportional changes in rainfall, maximum and minimum temperatures at the experimental site.

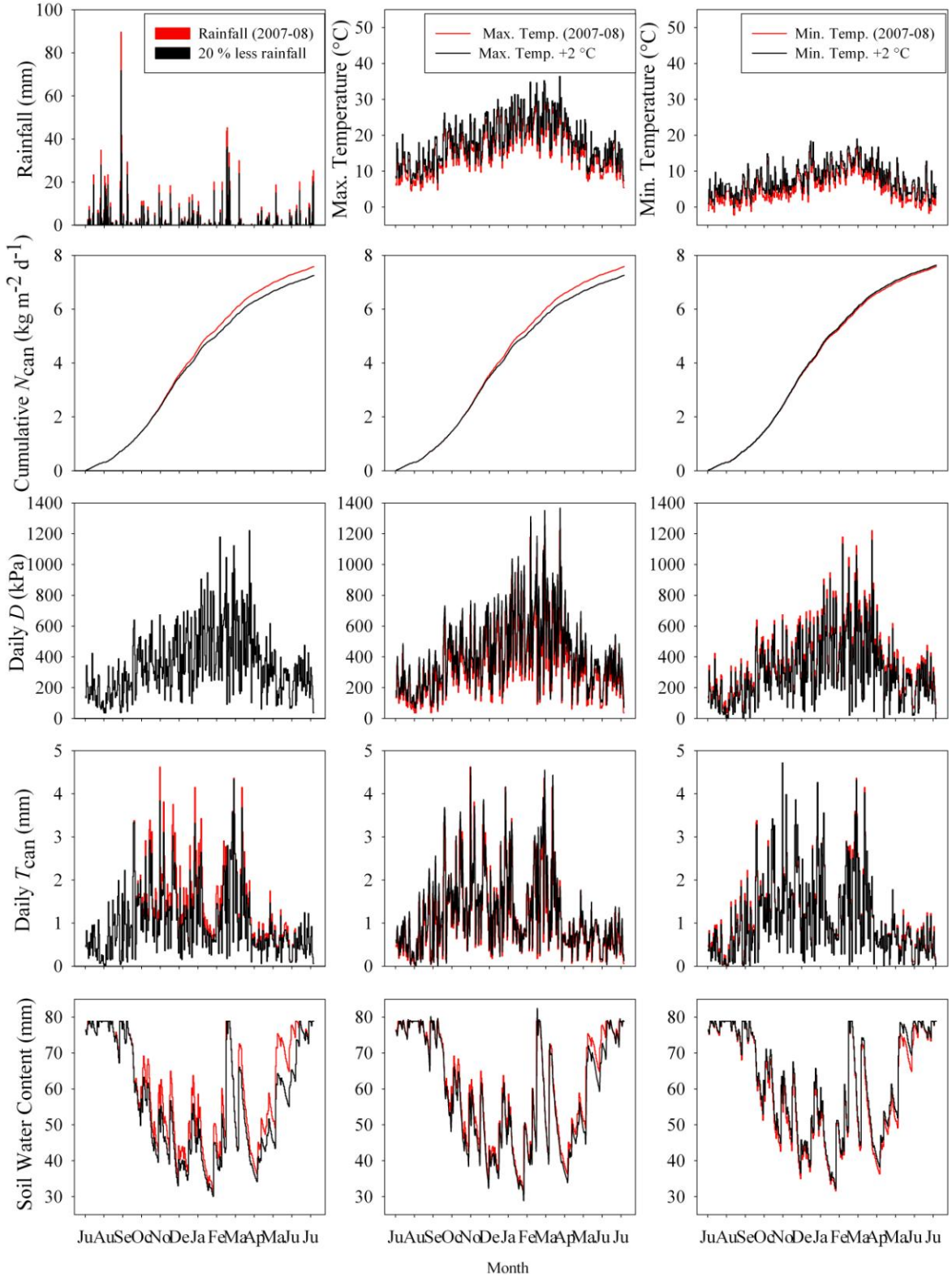

Figure 4.9 Influence of seasonal changes in rainfall, maximum temperature and minimum temperature on cumulative canopy net carbon exchange (N_{can}), daily vapour pressure deficit (D), daily canopy transpiration (T_{can}) and soil water content. Comparison of rainfall, maximum temperature and minimum temperature recorded in the field with 20 % less rainfall, 2 °C higher maximum and minimum temperatures are shown.

4.4 DISCUSSION

4.4.1 Response of canopy carbon exchange to seasonal changes in environmental variables at the field site

Gas exchange in the grass most strongly influenced seasonal variation in canopy photosynthesis and respiration, and therefore aboveground carbon exchange. The higher rate of canopy photosynthesis in the grass from late spring to early summer (Nov-Jan) was mainly due to canopy development, reflecting relatively high leaf area in response to changes in environmental variables i.e., greater solar radiation with moderate rainfall and adequate soil water content. The rate of canopy photosynthesis in the grass (23.6 Mg C ha^{-1} $year^{-1}$), was close to the calculated annual gross primary production (GPP) of an intensively grazed perennial rye grass growing on a similar soil type in New Zealand observed by Mudge (2009) using eddy covariance techniques - accounting 19.5 Mg C ha^{-1} $year^{-1}$. The contribution of canopy photosynthesis of kānuka was only 9.5 % of total canopy photosynthesis and the higher rate of canopy photosynthesis in kānuka was observed in summer. The differences in the timing of peak canopy photosynthesis observed in the grass and kānuka in relation to changes in environmental variables suggest that photosynthesis in the grass is high in conditions of high solar radiation and soil water content while photosynthesis in kānuka is highest at high radiation and relatively low soil water content. It is more likely that during the dry season, access to soil water is limited for the grass due to the shallower root system in comparison with kānuka.

Canopy respiration in the grass was high when active grass growth started in spring, but decreased in mid-summer and then increased again in autumn. Canopy respiration in kānuka was high in autumn and low in late winter. The lower rate of canopy respiration in the grass during summer was likely mediated by the effect of low soil water content while in kānuka, canopy respiration remained more or less constant during this period. The influence of foliar respiration of kānuka to total cumulative annual canopy respiration was evident from mid-spring to early

winter, accounting for 17.3 % of the total canopy respiration contributed by grass. The contribution of cumulative canopy respiration of kānuka to total canopy respiration was nearly twice that of its contribution of cumulative canopy photosynthesis to total canopy photosynthesis. The response of canopy respiration in both plant types to changes in environmental variables shows that canopy respiration responds most strongly to changes in ambient temperature and soil water content, and its response was much more rapid than that of photosynthesis. This speed of respiratory acclimation to changes in environmental variables is in agreement with several recent findings (Lee *et al.* 2005; Ow *et al.* 2010; Crous *et al.* 2011).

Seasonal changes in the ratio of R_{can} / A_{can} were limited, showing that carbon uptake was high in both the plant types from late spring to early autumn due to relatively high rates of canopy photosynthesis and low rates of canopy respiration. From late autumn to winter R_{can} / A_{can} ratio was high due to relatively high rates of respiration and low rates of photosynthesis. The proportional decline in the seasonal rate of canopy respiration in summer was slightly higher than the proportional decrease in canopy photosynthesis in grass. Therefore, the carbon loss by respiration did not exceed carbon gain by photosynthesis in both plant types during summer, unlike the response observed under severe drought conditions in *Acacia* sp. (Gimeno *et al.* 2010). In summer, thermal acclimation of canopy respiration was intensified by the influence of low soil water content while in winter, canopy respiration was higher than canopy photosynthesis (refer chapter-3), resulting in limited growth. The influence of low soil water content in accelerating respiratory acclimation was recently reported in *Eucalyptus saligna* (Crous *et al.* 2011). These findings together suggest that accounting for the combined effect of seasonal changes in temperature and soil water content on canopy respiration and canopy photosynthesis is important for determining the carbon balance in a grassland ecosystem when growth is limited in summer due to impaired photosynthesis.

4.4.2 Sensitivity of canopy carbon exchange to changes in photosynthetic and respiratory parameters

Sensitivity analysis of the model showed that canopy carbon uptake is strongly influenced by changes in the quantum efficiency of electron transport in both kānuka and the grass. Results showed a positive correlation of quantum efficiency of electron transport with canopy photosynthesis, canopy net carbon exchange, canopy light use efficiency and canopy water use efficiency. A strong impact of quantum efficiency of electron transport on canopy photosynthesis has been reported previously in coniferous forest canopies (Ibrom *et al.* 2006). Niinemets *et al.* (2001) showed that the quantum yield of electron transport is dependent on seasonal daily-integrated quantum flux density and may vary diurnally and seasonally due to changes in irradiance. Therefore, the sensitivity of photosynthesis to quantum yield of the electron transport in both kānuka and the grass suggests that for accurate assessment of canopy carbon uptake, it is important to determine the value of quantum yield of electron transport.

Proportional seasonal changes in the photosynthetic parameters V_{cmax} and J_{max} showed a positive correlation with canopy photosynthesis, canopy net carbon exchange, canopy light use efficiency and canopy water use efficiency. Previous studies showed that V_{cmax} and J_{max} are influenced by seasonal variations involving changes in various environmental factors such as temperature, soil water content (Wilson *et al.* 2000; Bernacchi *et al.* 2001; Bota *et al.* 2004; Grassi and Magnani 2005; Egea *et al.* 2011a) and phenological factors like leaf age, leaf width, leaf nitrogen content (Field and Mooney 1983; Reich *et al.* 1991; Wilson *et al.* 2000; Frak *et al.* 2001; Kitajima *et al.* 2002; Muraoka and Koizumi 2005; Egea *et al.* 2011a). It is evident from the sensitivity analysis that 15 % variation in V_{cmax} and J_{max} could result in nearly 4 to 6 % variation in estimated values of canopy photosynthesis and canopy net carbon exchange. Thus, incorporating seasonal changes in V_{cmax} and J_{max} in canopy carbon exchange models would

reduce the error induced by environmental and phenological factors on estimated values of canopy carbon uptake.

Sensitivity analysis of the model further showed that changes in respiratory capacity of kānuka and grass resulted in strong variation in estimated canopy respiration and in estimated net canopy exchange. Flanagan and Johnson (2005) reported an interacting effect of soil water content, temperature and plant biomass production on ecosystem respiration in a temperate grassland ecosystem, with a major influence of soil water content and above-ground biomass in seasonal and interannual variation in total ecosystem respiration. Several other studies have showed respiratory acclimation to seasonal changes in temperature (Atkin *et al.* 2000b; Atkin and Tjoelker 2003; Loveys *et al.* 2003; Atkin *et al.* 2005b; Lee *et al.* 2005; Armstrong *et al.* 2006a; Zhou *et al.* 2007; Ow *et al.* 2010) and daily changes in light (Searle *et al.* 2010). Overall, it is clear that seasonal changes in canopy respiration can strongly alter aboveground carbon balance. For this reason, accounting for the seasonal variation in canopy respiration in canopy carbon exchange models is essential to avoid erroneous estimates of canopy respiration.

4.4.3 Comparison of canopy carbon exchange using parameters measured under different growing conditions

The comparative analysis involving calculations of canopy carbon exchange of kānuka and grass using seasonally-acclimated parameters with instantaneous winter and summer parameters and those determined in the controlled growth cabinet experiment clarified the possible variations in estimating aboveground canopy processes under different growth conditions and time frames. When the model used photosynthetic and respiratory parameters measured under growth cabinet conditions, the estimated values of canopy photosynthesis were much lower than those based on seasonally-acclimated parameters. However, when the model considered the instantaneous response of grass and kānuka in winter and summer the estimated

value of canopy photosynthesis was relatively higher in winter than in summer. Further, both the instantaneous responses yielded values that were slightly higher than the seasonally-acclimated response. Differences in photosynthesis between controlled growth cabinet and field measurements have previously been reported in studies investigating photosynthetic responses to temperature (Gunderson *et al.* 2000; Wise *et al.* 2004). The modelling analysis comparing different growth conditions shows that under controlled conditions, the response of canopy photosynthesis to imposed static treatments differs from the response of canopy photosynthesis to naturally thermally fluctuating environments. Differences in response will also result from other uncontrollable natural factors e.g., changes in irradiance, drought and nutrient levels. The present study shows that considering instantaneous responses of photosynthetic or respiratory parameters could either underestimate or overestimate the simulated annual carbon gain or carbon loss due to seasonal changes in temperature or soil water content. Moreover, this analysis further suggests that when modelling annual canopy carbon exchange, it is important to consider seasonal responses and avoid using single instantaneous responses of photosynthetic and respiratory parameters in order to avoid large errors in modelled aboveground values of annual carbon gain or carbon loss within an ecosystem.

Ow *et al.* (2010) demonstrated the importance of considering respiratory acclimation in models to improve the validity of modelled responses. When comparing the estimated canopy respiration modelled using different experimental conditions, the seasonally-acclimated response corresponded closer to the instantaneous response in summer. However, the estimation of canopy respiration using an instantaneous winter response differed by nearly 40 % with respect to the seasonally acclimated response. Thus, modelling canopy respiration using respiratory measurements in cooler months could lead to overestimation of canopy respiration and substantial errors in net carbon uptake.

Even though there were dissimilarities in modelled monthly estimates of canopy respiration between the seasonally-acclimated and growth cabinet based measurements, the estimated cumulative canopy respiration using the growth cabinet response resembled the seasonally acclimated response fairly closely. There is good evidence that leaf respiration can acclimate to changes in ambient temperature over a short period of time (days), when grown under contrasting growth conditions (Atkin *et al.* 2000b; Bolstad *et al.* 2003; Lee *et al.* 2005; Ow *et al.* 2008b). However, frequent changes in seasonal factors may cause changes in the timing of acclimation, resulting in 'delayed' acclimation that has recently been reported in New Zealand alpine grass species (Searle *et al.* 2010). Therefore, it is appropriate to incorporate seasonally dynamic responses of respiration into carbon exchange models – these may improve the validity of model calculations of canopy carbon exchange in ecosystem models.

4.4.4 Predicting future climate impacts on canopy carbon exchange

Recent studies on changes in seasonal variation in CO_2 exchange in a grassland ecosystem have highlighted a combined influence of temperature and precipitation (Fu *et al.* 2009; Fay *et al.* 2011). Therefore, it is important to consider the influence of temperature and soil water content simultaneously when estimating canopy net carbon exchange. In the present study, the modelled response of canopy processes to proportional changes in rainfall and maximum and minimum temperature conditions displayed similar trends in A_{can}, L_{can} and N_{can}, while contrasting trends were observed between the responses of T_{can} and W_{can}. The response of R_{can} was unique, with a limited response to changes in rainfall but a strong response to changes in maximum and minimum temperatures. Changes in canopy photosynthesis due to rainfall were mainly associated with variation in canopy transpiration. Farquhar and Sharkey (1982) observed a negative relationship between rainfall and water use efficiency due to the influence of stomatal conductance limiting transpiration more than photosynthesis. Reduction in stomatal conductance

as a function of decreasing soil water content has also been reported for several forest trees (Kozlowski *et al.* 1991; Wilkinson 2000). Under scenarios modelling the effect of reduced rainfall, stomatal conductance may decline below its physiological maximum due to stomatal closure to internal water loss. This causes a strong reduction in canopy transpiration and canopy net carbon exchange. The negative relationship between rainfall and water use efficiency and the positive relationship between rainfall and leaf transpiration rate are also comparable with the finding of Niu *et al.* (2011), who reported a linear reduction in leaf scale water use efficiency with increasing rainfall. It was also observed that reducing rainfall had little influence on daily vapour pressure deficit, which was in agreement with the findings of Jarvis and McNaughton (1986) and Martin (1989) who found that the rate of transpiration in grass swards is less affected by vapour pressure deficit because it is mainly regulated by radiation interception.

The increased rate of transpiration could remove available soil water more rapidly resulting in lower water use efficiency. Moreover, it is likely that the higher rate of photorespiration due to increasing maximum temperature could result in reduced carboxylation efficiency, affecting canopy photosynthesis and canopy net carbon exchange. On the other hand, when minimum temperature is increased the rate of canopy transpiration declined because of the effect of slightly lower vapour pressure deficit on stomatal conductance. This is comparable with a previous study illustrating the response of transpiration to slightly higher nighttime temperatures (Kirschbaum 2004). The lower rate of transpirational water loss resulted in slightly higher soil water content and water use efficiency and that is reflected in a slight increase in canopy photosynthesis and canopy net carbon exchange. There is previous evidence that nighttime stomatal conductance can alter the rate of transpirational water loss in C_3 plants (Caird *et al.* 2007).

The rate of canopy respiration increased slightly with increasing maximum and minimum temperatures despite thermal acclimation in both plant types. Several previous studies have

demonstrated the influence of variation in annual precipitation and identified large variation in aboveground biomass production in grassland ecosystems on individual sites (Li *et al.* 2005; Gilmanov *et al.* 2007; Ma *et al.* 2007; Hussain *et al.* 2011). The present study, analysing the impact of seasonal variation in two major environmental variables individually on aboveground canopy processes, demonstrates that seasonal variation in canopy photosynthesis was mainly due to changes in rainfall, while seasonal variation in canopy respiration was mainly due to changes in temperature. The impacts of both rainfall and temperature changes on canopy net carbon exchange was evident, with relatively greater influence of air temperature.

The analysis so far has considered the influence of increasing temperature and decreasing rainfall separately and has also assumed that with global warming there would be the same increase in both daily minimum and maximum temperatures. However, according to the IPCC (2001), future temperature increases will likely involve greater increases at nighttime than during the daytime, with nighttime temperatures increasing at potentially twice the rate of daytime temperature. To illustrate the impact of increasing daytime and nighttime temperature on the above ground net carbon exchange, the model was run under different daytime and nighttime temperatures (nighttime temperature increased by nearly twice as much as daytime) and the response of canopy processes is shown in Figure 4.10. An increase in minimum and maximum temperature up to 2 °C at night and 1 °C during the day resulted in a gradual increase in canopy photosynthesis and canopy respiration. This is in agreement with other recent studies (Piao *et al.* 2008; Cai *et al.* 2010) and predictions of several modelling studies that calculate a higher release of carbon dioxide into the atmosphere due to climate change (Heimann and Reichstein 2008). In general, these findings uphold the basic physiological principle that the temperature optimum for photosynthesis occurs at a lower temperature than respiration and the optimum region of the photosynthesis-temperature response curve has a broad plateau (Larcher 2003).

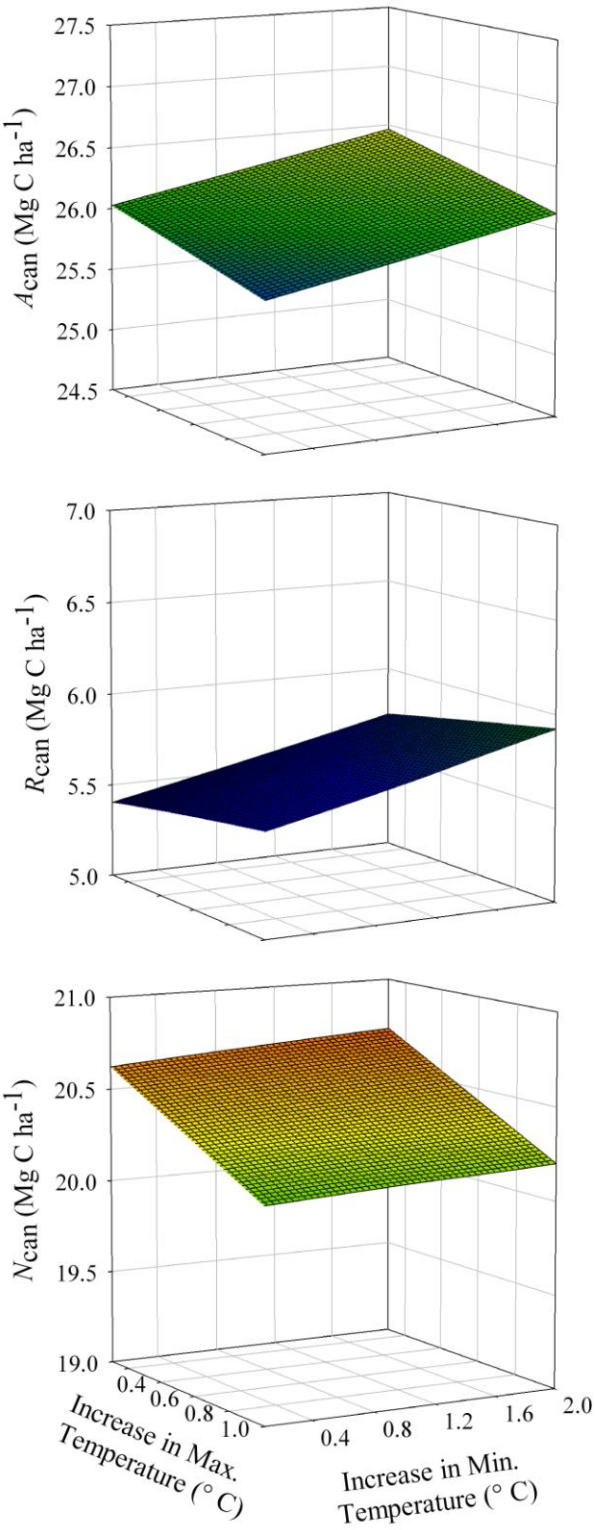

Figure 4.10 The response of canopy photosynthesis (A_{can}), canopy respiration (R_{can}) and canopy net carbon exchange (N_{can}) to increasing temperatures by manipulating nighttime minimum temperature at a rate twice the rate of daytime maximum temperature.

The response of canopy photosynthesis to increasing maximum and minimum temperatures was different from the response in canopy respiration. Canopy photosynthesis increased with increasing minimum temperatures and decreased with increasing maximum temperature. The reduction in canopy photosynthesis with increasing maximum temperature is likely associated with the response of stomatal closure due to higher vapour pressure deficits causing higher stomatal limitation of photosynthesis. The impact of warmer conditions in reducing canopy photosynthesis has been reported in previous studies (Roderick *et al.* 2001; Cai *et al.* 2009). The reduction in canopy photosynthesis combined with higher respiration resulted in reduced canopy net carbon exchange, which is consistent with other studies showing reduction in net carbon exchange due to seasonal warming (Piao *et al.* 2008).

The interactive effect of temperature and varying soil water content on canopy processes was also investigated by manipulating the rainfall from –20 to 8 % of the long-term average rainfall observed under field conditions under the varying temperatures simulated for Figure 4.10. The details of various temperature conditions manipulated in the model are given in Table 4.3. Each point on the resulting 3-D plot (Figure 4.11) shows the response of canopy photosynthesis, canopy respiration and canopy net carbon exchange to increasing temperatures and varying rainfall.

Table 4.3 Changes in temperature conditions used in the model integrating the combined effects of increasing temperatures and changing rainfall conditions as illustrated in Figure 4.11.

Condition	Increase in maximum temperature (°C)	Increase in minimum temperature (°C)
0	0	0
1	0	0.2
2	0.2	0.4
3	0.5	1.0
4	0.8	1.6
5	1	2

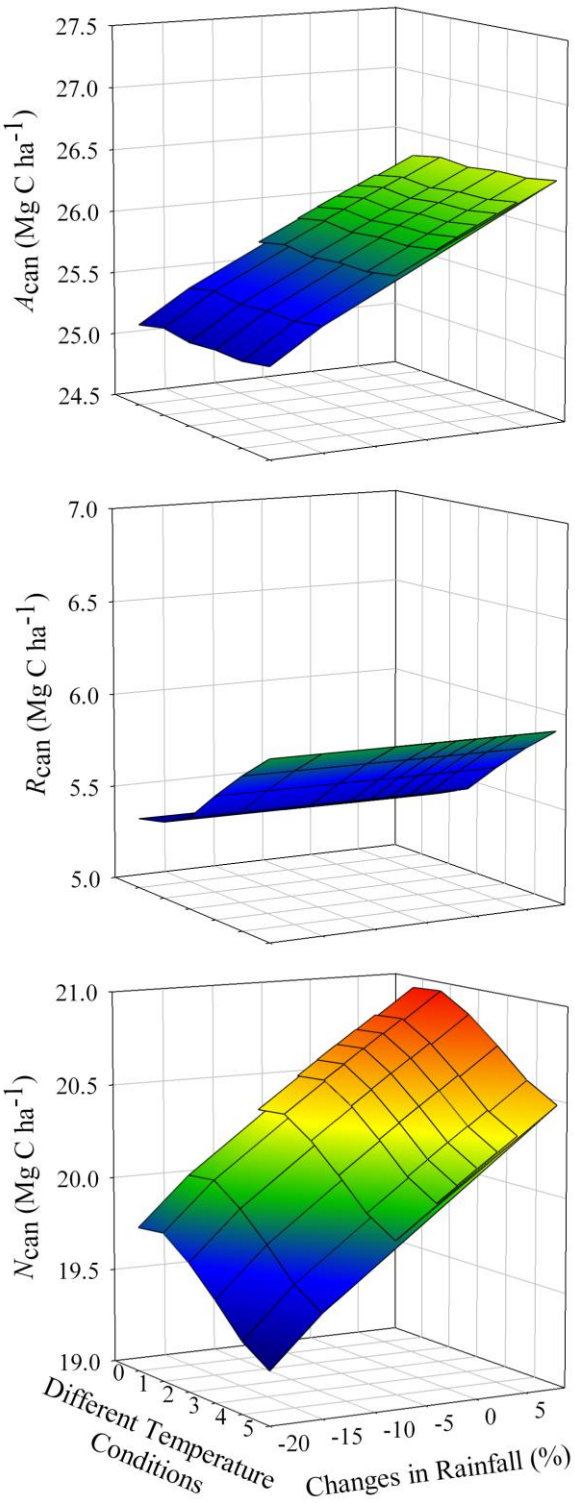

Figure 4.11 Simulation of the interactive effect of soil water content and temperature on canopy photosynthesis (A_{can}), canopy respiration (R_{can}) and canopy net carbon exchange (N_{can}) by manipulating rainfall pattern with a reduction from 20 % to an increase in 8 % and increasing nighttime temperature at a rate nearly twice the rate of daytime temperature. Changes in daytime and nighttime temperature are represented as different temperature conditions ranging from 0 - 5 and the details are in Table 4.3.

The influence of rainfall was much greater than the effect of increasing temperatures on canopy photosynthesis and canopy net carbon exchange (and *vice-versa* on canopy respiration). Reduction in rainfall drastically reduced canopy photosynthesis and canopy net carbon exchange while its effect on canopy respiration was less pronounced. In contrast, rising temperature slightly reduced canopy photosynthesis while it moderately influenced canopy respiration. When increasing temperature was accompanied by a concomitant reduction in rainfall, their combined effect resulted in a very strong reduction in canopy net carbon exchange. The present study shows how the interaction between rising temperature and varying soil water content is critical to plant responses to climate change. These findings are comparable with the results of Shaw *et al.* (2006), who found a significant increase in forest productivity at warmer site because of summer precipitation. Moreover, it is important to note that the effect of increasing temperature on canopy respiration is slightly lessened by the effect of reducing soil water content. The influence of drought on reducing the rate of plant respiration has been reported previously (Atkin and Macherel 2009; Crous *et al.* 2011). Therefore, accounting for the influence of reducing soil water content accompanied by increasing temperature may reduce the impact that increasing temperature might have on intensifying future climate impacts through increased plant respiration.

In conclusion, for the combinations of climate change scenarios observed to date and anticipated into the future, the present study informs us that aboveground canopy process are strongly influenced by changes in temperature and rainfall conditions. It is clear that increasing temperatures influence above ground canopy processes, however the extent and direction of this temperature effect is also strongly influenced by changes in soil water content. The different response of aboveground canopy carbon exchange to varying temperature and rainfall conditions has important implications for plant carbon balance. This study indicates that the temperature dependence of above-ground canopy processes should not be assumed and caution should be

taken when considering short-term or single instantaneous seasonal responses of above ground processes for modelling canopy carbon exchange of an ecosystem.

4.5 SUMMARY

The present study used the net ecosystem exchange model (NEEMo), which integrates the response of plant physiological measurements to seasonal variations in environmental variables incorporating plant and coupled climate-carbon simulations, to investigate aboveground canopy carbon uptake in grassland reverting to shrubland. The model estimated aboveground canopy photosynthesis and canopy respiration as 26 Mg C ha^{-1} and 5.4 Mg C ha^{-1} (respectively) and the relative contribution of grass to these canopy processes was nearly 10-fold and 5-fold higher than the shrub (kānuka), respectively. Sensitivity analysis of the model in predicting annual estimates of canopy variables showed a greater influence of the quantum efficiency of electron transport (α) over monthly measurements of photosynthetic parameters (V_{cmax} and J_{max}) and R. Furthermore, the model identified the significance of considering seasonal changes in respiratory and photosynthetic parameters in estimating canopy carbon exchange and pointed out possible errors in using these parameters estimated under different experimental conditions. To understand the response of canopy carbon exchange to future climate change scenarios, daytime maximum and nighttime minimum temperatures and rainfall were manipulated separately and simultaneously. The influence of changes in these environmental variables on canopy carbon exchange was strong when considered separately and in combination. The present study shows that ecosystem models integrating plant physiological measurements with major environmental variables can quantify precisely above-ground carbon exchange and resolve the complex issues of simultaneous interactions of varying environmental driving variables on canopy carbon exchange.

CHAPTER 5

Discussion and future directions

Chapter 5

5.1 THESIS OBJECTIVES

This thesis investigated the response of photosynthetic and respiratory parameters and their acclimation to varying temperature and soil water conditions in two different plant types (pasture grass and the native shrub, kānuka). The controlled environment study allowed for the investigation of the effects of short-term changes in growth temperature and soil water content (θ) on the response of photosynthesis and respiration. By contrast, the field study facilitated the investigation of the effects of continuous medium- and long-term changes in temperature and soil water content on the acclimation of both processes to natural diurnal and seasonal changes. Finally, the modelling study developed predictions of the impacts of future changes in temperature and soil water content and their interactions on canopy photosynthesis, canopy respiration and canopy net carbon exchange. With these three different approaches, I have attempted to address the following questions:

1) How do photosynthetic and respiratory parameters respond to concurrent changes in growth temperature and soil water content?

2) What is the importance of varying soil water content on the thermal acclimation of photosynthesis and respiration?

3) What are the likely impacts of rising temperature and reducing rainfall on canopy carbon exchange?

5.2 SUMMARY AND DISCUSSION OF KEY FINDINGS

5.2.1 Response of photosynthesis to changes in growth temperature and soil water content under growth cabinet and field conditions.

- Photosynthetic capacity of kānuka and the grass declined significantly when soil water content fell below near 20 % in the short-term growth cabinet experiment. The temperature sensitivity of photosynthesis was low at low soil water content and increased at moderate to high soil water content in both the plant types.
- In the grass, seasonal responses of photosynthetic parameters to changes in temperature and soil water content were significant and photosynthetic rate remained relatively high when soil water content and temperature were moderate. Photosynthetic parameters in the grass declined significantly when soil water content fell below 20 %, while the response of kānuka to seasonal changes in soil water content was not significant.

Under growth cabinet conditions, the plants were exposed to controlled temperature and light levels for nearly two weeks and then they experienced a progressive reduction in soil water content. The photosynthetic capacity of the grass and kānuka was relatively less in the growth cabinet compared to similar soil water content under field conditions. Under field conditions, these plants experienced long-term changes in temperature and soil water content and they are exposed to a wide range of environmental variations. Statistical analysis showed that photosynthetic capacity of the grass was sensitive to both short-term and long-term changes in temperature and soil water contents but kānuka became less sensitive to soil water content under field conditions. Photosynthetic rates in the grass were high in spring but declined in summer and then recovered upon receiving autumn rainfall, then declined further in winter. In kānuka,

the photosynthetic rate in autumn was nearly three times higher than that at other times in the year and displayed similar sensitivity to the grass to very low and very high soil water content. In summer, photosynthetic inhibition was due mainly to a reduction in soil water content and then recovery of photosynthesis occurred in both plant types after receiving autumn rainfall, which has been reported in other evergreen species (Vaz *et al.* 2010).

The temperature sensitivity of photosynthetic parameters increased with increasing soil water content and optimum soil water content was around 30-35 % in both plant types under growth cabinet conditions. The sensitivity of photosynthetic parameters in the grass to soil water content was similar under both growth cabinet and field conditions. Statistical analysis revealed that the temperature sensitivity of photosynthetic parameters declined below 20 % soil water content in both plant types in the growth cabinet experiment, while the reduction in temperature sensitivity of kānuka under field conditions was predominantly due to changes in temperature. It is noteworthy that kānuka is capable of utilising soil water efficiently and its deep roots can access available soil water from lower soil layers than the shallow fibrous roots of the grass. It is clear from the field experiment that the two plant types growing under similar conditions had different sensitivities to soil water conditions.

5.2.2 Response of respiration to changes in growth temperature and soil water content under growth cabinet and field conditions.

- Under growth cabinet conditions, respiratory capacity (R_{10}) increased with increasing soil water content in kānuka and declined significantly when the value of soil water content fell below 15 %. There was no significant influence of soil water content on respiratory capacity in the grass. Under field condition, respiratory parameters were highly sensitive to soil water content. The temperature sensitivity of respiration (E_o) declined with increasing soil water content in both plant types.

- A higher degree of respiratory acclimation was observed in response to seasonal changes in temperature in the field than under growth cabinet conditions. Seasonal changes in soil water content had a strong influence on the process of respiratory acclimation and its effect was consistent in both plant types.

In the growth cabinet experiment, rates of respiration were low while under seasonally variable field conditions respiration rate was high. The difference in the respiration rate was likely associated with a direct relationship to irradiance and photosynthesis (Whitehead *et al.* 2004a). Under field conditions, respiratory parameters were more sensitive to soil water content in both the plant types. When plants assimilate more carbon in response to relatively high temperature and irradiance, the rate of respiration may also increase, because photosynthesis and respiration are interdependent. For example, Whitehead *et al.* (2004a) have shown that the rate of night-time leaf respiration is highly dependent on total photosynthesis of the previous day, demostrating a direct and rapid link between photosynthesis and respiration. The strong link between photosynthesis and respiration has also been reported from several other studies (Gifford 2003; Loveys *et al.* 2003; Hartley *et al.* 2006). In the growth cabinet experiment, respiration was limited by substrate supply and thus respiration did not change significantly in response to short-term changes in temperature and soil water conditions. Overall, the dependence of respiration on the rate of photosynthesis may likely place a strong constraint on the capacity of respiration to respond to reduced soil water content. This indicates that caution should be taken when interpreting responses from growth cabinet experiments and comparing them to field situations.

Under field conditions, a shift in the elevation of the temperature response of respiration (higher in winter and lower in summer; 'Type II acclimation' *sensu* Atkin & Tjoelker (2003)) resulted in somewhat similar rates of dark respiration across a wide range of minimum

temperatures in both the plant types. This finding concurs with previous reports and provides strong evidence of respiratory acclimation to diurnal and seasonal temperature change (Ow *et al.* 2010). Moreover, in both plant types, a downward shift in E_o values was also observed – this is indicative of 'Type I acclimation' - but this short-term change in E_o was more predominant in the grass. Reduction in E_o was observed mostly in summer and in late winter to mid spring in both plant types. The greater reduction in E_o in the grass in summer was due predominantly to a greater impact of water stress on the grass than on kānuka. Under field conditions, reduction of Q_{10} (E_o) has been reported in several evergreen plants in summer (Paembonan *et al.* 1991; Atkin *et al.* 2000b; Tjoelker *et al.* 2009). During late winter to mid-spring, when grass grew quickly in response to higher soil water content and warmer conditions, the fast growing leaf tissue was in transition from young to mature. This observation is in agreement with a previous report that showed an increase in Q_{10} value throughout the transition from immature to mature tissue (Marra *et al.* 2009). Seasonal changes in soil water content had an important role in regulating respiration, particularly when the soil water content fell below a threshold value, θ_c. However, when soil water content was above a threshold value of θ_c (Table 2.4 and 3.4), the rate of respiration was likely to be associated with changes in developmental stage and biochemical activity (involving changes in substrate availability, ATP demand, respiratory pathways, respiratory enzyme capacity or adenylate limitations) in response to changes in other environmental factors.

5.2.3 Response of modelled canopy carbon exchange to changes in temperature and soil water content.

- Modelled canopy photosynthesis and respiration were sensitive to changes in rainfall and maximum and minimum temperature.

- A combination of seasonal variation in both photosynthetic and respiratory parameters in response to changes in rainfall and temperature is required to model canopy net carbon exchange effectively.

The grass canopy predominantly regulated canopy photosynthesis and canopy respiration of this pasture-to-shrubland regeneration site. Sensitivity analysis of the modelling parameters showed a strong impact of the quantum efficiency of electron transport followed by V_{cmax} and J_{max} on canopy water use efficiency and light use efficiency. Reductions in rainfall resulted in reductions in canopy photosynthesis in association with reductions in canopy transpiration that is regulated predominantly by changes in stomatal conductance. It was clear from statistical analysis that increases in temperature can increase canopy photosynthesis if the soil water content is above 20 %. Canopy respiration responded to increasing temperature, but importantly, acclimation of canopy respiration to ambient temperature can dampen the predicted increase in respiration (King *et al.* 2006; Reich 2010). Statistical analysis of field data further revealed the influence of soil water content in regulating the acclimation of canopy respiration in both plant species. It is clear that respiration at any given time will be regulated by both thermal and soil water content responses.

5.3 RECOMMENDATIONS FOR FURTHER STUDIES

Improved understanding of the interaction of soil water content and temperature on photosynthesis is needed. Since future climate change scenarios predict increased prevalence of drought in certain areas, investigation of the interactions between soil water content and temperature and their impacts on photosynthetic parameters could usefully be extended. Previous studies have shown that under mild to moderate water stress, photosynthetic recovery is much faster than under severe water stress (Quick *et al.* 1992; Flexas *et al.* 1999). Furthermore, studies

on the interaction between soil water content and temperature could also be extended to regions facing more frequent drought and include more detailed investigation of photosynthetic acclimation to repeated dry and wet cycles. Understanding of stomatal and non-stomatal limitations to the recovery of photosynthesis following drought is scarce, and such studies will enhance our understanding of water use efficiency and improve the efficiency of predictions of ecosystem productivity in regions that are already under drought and are likely to be more prone to drought in the future.

Respiratory acclimation to seasonal changes in temperature has now been observed in several studies. However, a clear understanding of the roles of other environmental factors on respiratory acclimation is needed to model precisely the response of respiration to environmental changes. The results of Chapter 3 showed a strong relationship between the respiratory parameters and soil water content. Moreover, statistical analysis showed that respiratory parameters became relatively more sensitive to soil water content in both plant types under field conditions. Further research is essential to understand the role of soil water content in regulating the speed of respiratory acclimation and to investigate the mechanisms regulating the rapidity of respiratory acclimation under varying soil water contents. This issue could be resolved in growth cabinet studies by subjecting plants to a particular temperature under steady state or fluctuating soil water content.

To predict future canopy carbon storage and exchange in a developing shrubland, additional information on growth rate, phenological and physiological measurements and empirical relationship between biomass and diameter of kānuka at different ages is needed. Photosynthetic parameters may change with age when kānuka grows and accumulate more biomass or foliar nitrogen. Such details may provide the basis for modelling changes in land-cover/use when pastureland reverts to woody vegetation. Therefore, study must be extended to analyse the age-related changes in photosynthetic and respiratory parameters in kānuka, as it

becomes a more prominent component in the reverting shrubland. For assessing the spatial and temporal changes in carbon exchange in a developing shrubland, and for scaling these processes to the landscape level, application of remote sensing techniques would be extremely helpful.

5.4 IMPLICATIONS OF THE RESPONSE TO CLIMATE CHANGE FOR THE CANTERBURY REGION

Based on previous reports detailing climate change scenarios for New Zealand, drought risk is projected to increase during this century in all areas that are currently drought-prone (Mullan *et al.* 2005). By 2090, droughts are projected to occur in several regions, including Canterbury, and the frequency of droughts is projected to occur more than four times as often. To put this into some context, in this final section I report on the possible changes in future canopy carbon exchange in a shrubland that is in the early stage of reversion from pastoral land in response to the predicted highest IPCC emission scenario (A1F1) (IPCC 2007). For the Canterbury region, NIWA (2008) has predicted an increase in maximum temperature of 1.9 and 5.0 °C and minimum temperature of 0.5 and 1.6 °C by 2040 and 2090, respectively. Based on climate change scenario data by the Ministry for the Environment (MfE) (2009) and Reisinger *et al.* (2010), the annual average precipitation across the Canterbury region is expected to reduce by nearly 2 and 3 % for 2040 and 2090 under A1F1 scenario. If we compare the predicted net carbon exchange under present conditions with that under the future A1F1 scenario, we see that it decreases by nearly 6 % by 2040 and 17 % by 2090 as a result of a 2.8 % and 8 % reduction in canopy photosynthesis and nearly 9 % and 26.7 % increase in canopy respiration by 2040 and 2090, respectively (Figure 5.1). The stimulatory effect of increasing temperature and decreasing rainfall on respiration was much greater than that on photosynthesis and this resulted in a reduction in annual canopy net carbon exchange. Drought induced reductions in photosynthesis combined with increased respiration in response to higher temperatures (causing reduction in net

carbon uptake) have been reported previously (Piao *et al.* 2008). If the trend for a greater stimulation of respiration than photosynthesis continues in response to increasing temperature and reducing rainfall conditions (the A1F1 scenario), the annual net carbon exchange of the pasture-shrub system would likely decline in the future.

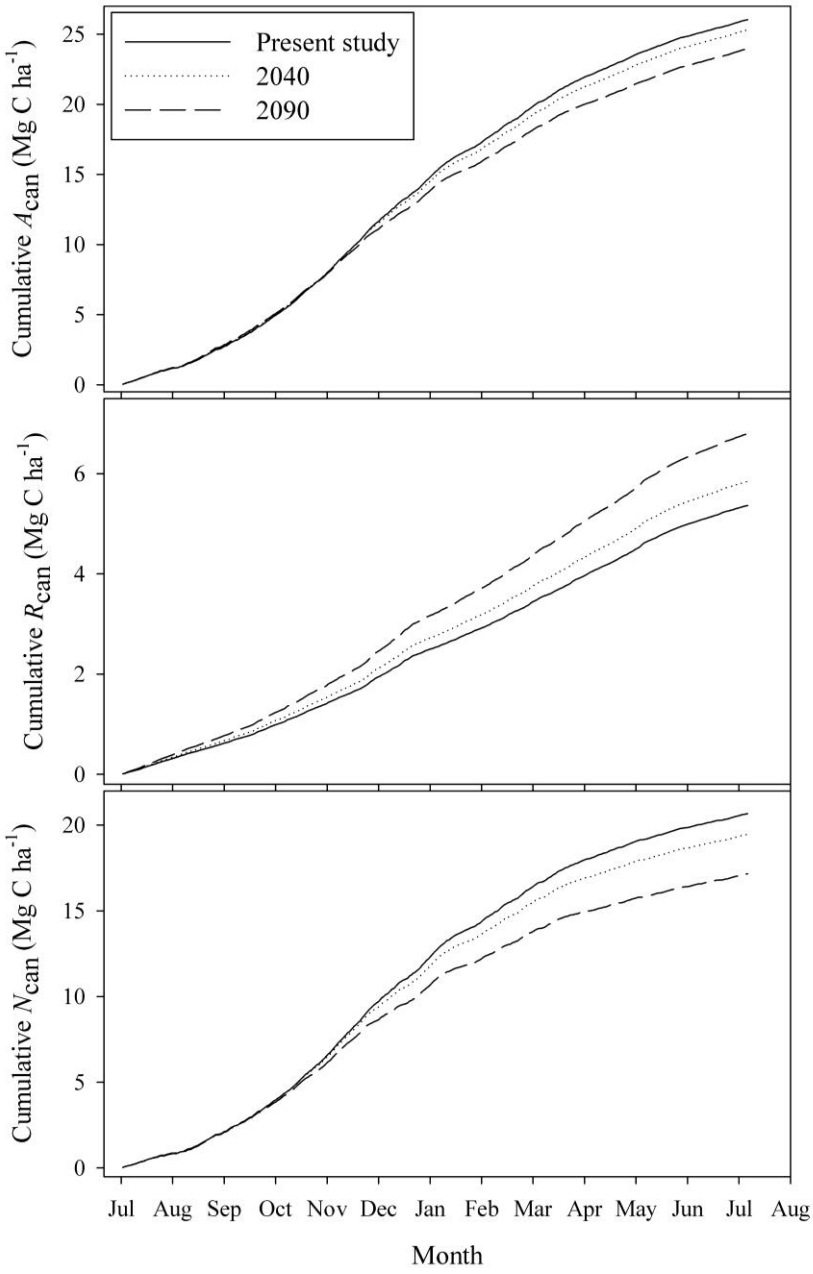

Figure 5.1 Changes in modelled seasonal cumulative canopy photosynthesis, A_{can}; canopy respiration, R_{can} and canopy net carbon exchange, N_{can} of a developing shrubland in the present condition and in response to the future climate change (A1F1 scenario) predicted for 2040 and 2090.

5.5 CONCLUDING STATEMENT

This study has focused on leaf-level responses of two key metabolic processes in two different plant types to changing growth temperature and soil water conditions and analyses the impact of these on above-ground carbon exchange of a system undergoing reversion from pasture to shrubland. This study has highlighted the effect of soil water content and identified its interaction with temperature on carbon exchange processes. Results showed that at higher temperatures, when soil water content falls below a threshold value of approximately 20 %, the rate of photosynthesis is affected mainly *via* a reduction in photosynthetic capacity that results in reduced supply of photosynthetic assimilates for respiration. However the sensitivity of photosynthetic and respiratory parameters to soil water content is species-specific and varies according to the range of temperature and soil water content experienced by the plants. This study has further demonstrated that the sensitivity of respiratory parameters to soil water content may change under different growth conditions (growth cabinet vs. field), potentially mediated *via* leaf carbohydrate content. Importantly, the extent of acclimation to changing temperature and soil water content differed significantly between the pasture grass and kānuka. Understanding the response of carbon exchange processes to concurrent changes in multiple environmental variables and modelling the combined response of different plant types growing together in regenerating shrubland has important implications for accurate assessment of carbon storage particularly in the context of global climate change.

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